

November 15, 2022

Petition submitted via e-mail and UPS

PETITION TO REQUIRE HEALTH AND ENVIRONMENTAL TESTING AND
REGULATION ON POLYVINYL ALCOHOL UNDER THE TOXIC SUBSTANCES
CONTROL ACT AND THE REMOVAL OF POLYVINYL ALCOHOL FROM THE EPA'S
SAFER CHOICE AND SAFER CHEMICAL INGREDIENTS LISTS

Michael S Regan, Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W. Washington, D.C. 20460
Regan.Michael@epa.gov

Dear Administrator Regan,

Please accept the following petition on behalf of Blueland and Plastic Pollution Coalition joined by leading nonprofit organizations fighting plastic pollution and climate change; Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, 5 Gyres, GAIA (Global Alliance for Incinerator Alternatives), Oceanic Global Foundation, The Last Beach Cleanup, Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth, Surfrider and Made Safe. This petition is submitted under section 21 of the Toxic Substances Control Act and the EPA Safer Choice Standards and Safer Chemical Lists from the EPA Safer Choice Program. This petition requests that the EPA conduct requisite human and environmental health and safety testing for Polyvinyl Alcohol, also known as PVA or PVOH as it is used in consumer-packaged goods, with particular attention to the use of PVA in laundry and dishwasher detergent pods and sheets. The petition also requests that until such testing is completed, the EPA remove polyvinyl alcohol from its Safer Choice Program in order to curb plastic pollution.

Thank you for your consideration.

Signed on behalf of our BlueLand, Plastic Pollution Coalition and our co-signed partners.

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Section 1: Executive Summary

This petition is filed under section 21 of the Toxic Substances Control Act (TSCA) and the Safer Choice Standards and Safer Chemical Lists from the EPA Safer Choice Program¹. The petition requests that the Environmental Protection Agency (EPA) require health and environmental safety tests of Polyvinyl Alcohol (PVA, also known as PVOH), as it is used as a plastic film in consumer-packaged goods and used in all dishwasher and laundry pods and sheets. This petition also requests that PVA be removed from the Safer Choice List and Safer Chemical Ingredients List until the EPA can complete the requested health and environmental safety testing. PVA is a synthetic, petroleum-derived polymer that is theorized to contribute to plastic pollution in oceans, waterways and soil, and recent research suggests that it may negatively impact ecosystems and the food and water supply.² PVA also has the potential to exhibit bioaccumulative properties that could carry toxic chemicals and carcinogens up through the food chain.³ A recent study has even showed Polyvinyl Alcohol particles to be present in human breastmilk.⁴

PVA has many applications, but one fast growing use for PVA, and particular concern for the EPA Safer Choice program, is its use in dishwasher and laundry detergent pods and sheets. Detergent pods are wrapped in a thin layer of PVA plastic film and detergent sheets are woven together with PVA. Laundry and dishwasher pods are a popular format for consumers, with over 20 billion PVA wrapped laundry and dishwasher pods used every year in the U.S. alone. Regardless, few consumers realize that the PVA film surrounding pods is actually a petroleum-based plastic.⁵ For many, once the plastic pod is put into the washing machine or dishwasher, it is out of sight and out of mind; however, there are potential health and environmental implications. Consumers look to initiatives like the EPA Safer Choice program to evaluate whether their products are safe for themselves and the environment.⁶ EPA Safer Choice analyzes ingredients, product performance and packaging to ensure that products with an EPA Safer Choice label are safer for individuals and pets, as well as workers health and the environment.⁷

¹ 15 U.S.C. 53 §2603. Testing of chemical substances and mixtures
<http://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim>

² Rolsky C, Kelkar V. Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate. *International Journal of Environmental Research and Public Health*. 2021; 18(11):6027.
<https://doi.org/10.3390/ijerph18116027>

³ *Ibid.*

⁴ Ragusa, Antonio, Valentina Notarstefano, Alessandro Svelato, Alessia Belloni, Giorgia Gioacchini, Christine Blondeel, Emma Zucchelli, et al. "Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk." *Polymers* 14, no. 13 (June 30, 2022): 2700. <https://doi.org/10.3390/polym14132700>.

⁵ Rolsky C, Kelkar V. 2.

⁶ US EPA, OCSPP. "Safer Choice Standard and Criteria." Overviews and Factsheets, March 7, 2014.
<https://www.epa.gov/saferchoice/standard>

⁷ *Ibid.*

PVA is currently on the Safer Choice Program's Safe Ingredients List, suggesting to consumers that the PVA plastic film encasing laundry and dishwasher pods is safe for people and the environment, and does not have any adverse impacts on the planet. Nevertheless, research shows that ~75% of plastic pods just from laundry and dishwasher detergents remain intact throughout conventional, wastewater treatment, and may persist in our environment, waterways, oceans and soils.⁸ PVA is a water-soluble thermoplastic polymer that has the ability to biodegrade in a set of specific conditions and, when used in washing machines and dishwashers, is designed to go down drains.⁹ PVA dissolves and is flushed into municipal wastewater but does not fully biodegrade due to the conditions in most wastewater treatment plants (WWTPs).¹⁰ From there, it flows from municipal wastewater into our water systems and soils.¹¹ For PVA to completely biodegrade, it requires extremely specific conditions to be met in WWTPs, including a particular length of time spent in WWTPs and the presence of certain, acclimated microorganisms needed to degrade PVA completely.¹² If all conditions are not met, intact PVA plastic is released into wastewater effluent which goes into soil, waterways, oceans and beyond. Since these conditions are rarely met, if ever, studies estimate that over ~75% of intact PVA plastic is released back into the environment¹³. Given PVA's potential for persistence, and the unknown impacts it has on the environment, PVA should be removed from the Safer Choice Program's Safe Ingredients List until extensive health and environmental safety testing can be completed, and PVA's effect on the environment is determined.

Section 2: The Petitioners

This petition is submitted by Blueland and Plastic Pollution Coalition, and co-signed by Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, 5 Gyres, GAIA (Global Alliance for Incinerator Alternatives), Oceanic Global Foundation, The Last Beach Cleanup, Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth, Surfrider and Made Safe. Blueland is a consumer-packaged goods company that aims to eliminate single-use plastic packaging from everyday products, including cleaning and personal care products. Plastic Pollution Coalition is a non-profit communications and advocacy organization that collaborates with an expansive global alliance of organizations, businesses, and individuals to create a more just and equitable world free of plastic pollution and its toxic impacts. Beyond Plastics, Plastic Oceans International, The Shaw Institute, Lonely Whale, GAIA (Global Alliance for Incinerator Alternatives), Oceanic

⁸ Rolsky C, Kelkar V. 1

⁹ Doble, Mukesh, and Anil Kumar. "CHAPTER 9 - Degradation of Polymers." In *Biotreatment of Industrial Effluents*, edited by Mukesh Doble and Anil Kumar, 101–10. Burlington: Butterworth-Heinemann, 2005. <https://doi.org/10.1016/B978-075067838-4/50010-5>.

¹⁰ *Ibid.* 1

¹¹ *Ibid.* 1

¹² *Ibid.* 1

¹³ *Ibid.* 9

Global Foundation, The Last Beach Cleanup Rio Grande International Study Center, Inland Ocean Coalition, Occidental Arts and Ecology Center, Turtle Island Restoration Network, Friends of the Earth, Surfrider and 5 Gyres are non-profit organizations dedicated to eliminating plastic pollution and protecting the planet from the harms of plastic and other destructive activities on the environment. Made Safe is a third-party certification organization that aims to help businesses achieve high standards in their practices and with their products as it relates to human and environmental health.

Section 3: Relevant Background Information on Plastic Pollution

In the last 30 years, plastic consumption has quadrupled.¹⁴ In 2019, 2.6 million tons of plastic waste ended up in oceans and waterways.¹⁵ Studies show by 2050, there will be more plastic, by weight, in the ocean than fish.¹⁶ Plastic pollution can inflict substantial harm to aquatic and marine environments – globally, 800 species of animal are impacted by marine debris, 100% of marine turtle species have been found to ingest plastic pieces along with 59% of whale, 36% of seal and 40% of seabird species examined.¹⁷

Microplastics are tiny pieces of plastic debris. There is no universally agreed upon size definition for microplastics, though many use plastics less than 5mm in diameter as the guidance.¹⁸ Microplastics have been linked to environmental and human health concerns.¹⁹ Recently, microplastics have been found in the human bloodstream and even in human placentas and breastmilk.²⁰ These tiny pieces of plastic, as well as nanoplastics (which are even smaller), are recognized as a harmful ocean contaminant.²¹ Due to their tiny size, they are easily ingested by marine animals, and they also have been found in human food and water sources.²²

Section 4: PVA Treatment, Pollution and Potential Harms on The Environment

¹⁴ “Plastic Pollution Is Growing Relentlessly as Waste Management and Recycling Fall Short, Says OECD.” Accessed July 21, 2022. <https://www.oecd.org/newsroom/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm>.

¹⁵ *Ibid*.

¹⁶ *Ibid*.

¹⁷ MBRCtheocean. “Shocking Plastic Statistics.” Accessed July 21, 2022. <https://www.mbrctheocean.com/pages/shocking-plastic-statistics>.

¹⁸ US Department of Commerce, National Oceanic and Atmospheric Administration. “What Are Microplastics?” Accessed July 22, 2022. <https://oceanservice.noaa.gov/facts/microplastics.html>.

¹⁹ *Ibid*.

²⁰ Ragusa, Antonio, Alessandro Svelato, Criselda Santacroce, Piera Catalano, Valentina Notarstefano, Oliana Carnevali, Fabrizio Papa, et al. “Plasticenta: First Evidence of Microplastics in Human Placenta.” *Environment International* 146 (January 2021): 106274. <https://doi.org/10.1016/j.envint.2020.106274>.

²¹ *Ibid*.

²² Lim, XiaoZhi. “Microplastics Are Everywhere — but Are They Harmful?” *Nature* 593, no. 7857 (May 4, 2021): 22–25. <https://doi.org/10.1038/d41586-021-01143-3>.

PVA is a synthetic, petroleum-based plastic polymer found in many, everyday products and has become a popular plastic used in cleaning and personal care products.²³ PVA is often found in household items as a thin plastic wrapping, encasing single-dose detergents or woven into laundry detergent sheets. PVA is also increasingly being used in other cleaning and personal care products, such as body washes and toilet cleaners, and is designed to go down drains and into water systems. A recent study published in the *International Journal of Environmental Research and Public Health* titled, “Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate”, shows that while PVA does solubilize, it does not necessarily biodegrade. In the U.S., an estimated 61% of PVA goes to WWTPs, and an estimated 37% remains untreated. In total, Rolsky and Kelkar estimate that ~75% of PVA from dishwasher and laundry pods persists through conventional wastewater treatment, passing into waterways and ecosystems beyond.²⁴ In addition to environmental persistence, similar to other petroleum-based plastic, PVA could bioaccumulate and has the potential to absorb dangerous contaminants and move them up the food chain. This requires further research.²⁵

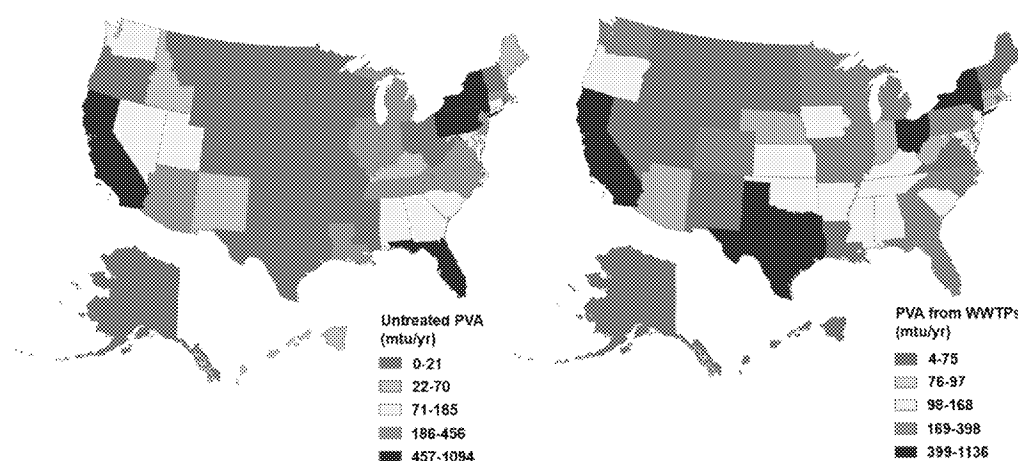


Figure 3. from *Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate* showing the amount of treated and untreated PVA released into the environment in the U.S. in metric ton unit/ year

PVA Degradability Process

PVA from laundry and dishwasher pods and sheets goes from consumer households to WWTPs. Dissolved PVA enters WWTPs but ~75% exits WWTPs intact, which is thought to pollute waterways and soil beyond.²⁶ This estimate is based on a conventional WWTP process. Facilities

²³ Rolsky and Kelkar, “Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate.” 2

²⁴ *Ibid.* 1

²⁵ *Ibid.* 12.

²⁶ *Ibid.*

with advanced treatment processes may have different degradation rates. PVA goes through the following phases in conventional wastewater treatment plants:

Phase 1: Primary treatment:

In the primary treatment phase, large solids are separated from the wastewater that has entered the facility. In this phase, dissolved PVA is not typically separated from the wastewater due to its hydrophilic properties.²⁷

Phase 2: Secondary treatment

If PVA were to biodegrade, it would likely take place in the secondary treatment phase. During the secondary treatment phase, PVA interacts with bacteria and microbes that typically break down biological waste in the wastewater. In conventional WWTPs within the U.S., specific PVA-adapted bacteria and microbes are needed to aid in the near to complete degradation of PVA, though they are not likely present.²⁸

If those microbes are present, the PVA in wastewater rarely encounters the microbes for long enough for the PVA to fully degrade. It is estimated that PVA needs to interact with the proper microbes for seven days, and typically wastewater remains in a conventional WWTP for two to three days.²⁹

Phase 3: Tertiary treatment

Once the wastewater has gone through the secondary treatment phase, the final phases are disinfection and filtration. In the disinfection phase, it is possible for remaining PVA to be degraded.³⁰ However, the technology to execute that degradation is expensive, and hence assumed to be rarely employed in WWTPs within the U.S.³¹

After the three treatment phases, wastewater is released from WWTPs. According to the study, PVA can travel into the environment via effluent or released within biosolids. If released with water, it has the potential to impact our waterways and environment. If released within biosolids, it will travel into landfills, onto soils or be incinerated.³²

²⁷ *Ibid*

²⁸ *Ibid.*

²⁹ *Ibid*

³⁰ *Ibid*

³¹ *Ibid.*

³² *Ibid.*

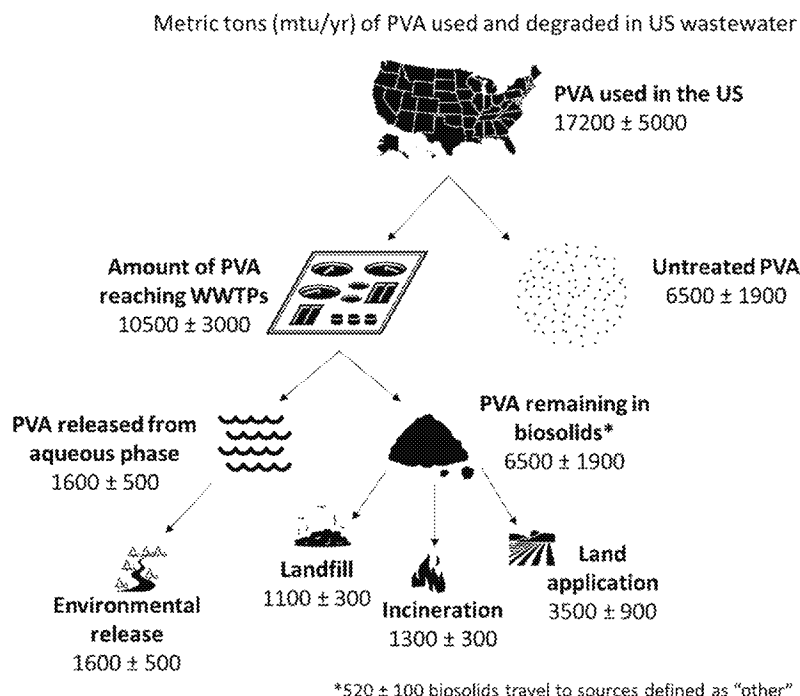


Figure 4 from *Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate* showing the path of PVA

The ~75% of PVA that remains intact after it leaves the WWTP weighs roughly eight thousand metric tons and is released back into the environment each year, just from plastic laundry and dishwasher pods alone.³³ With PVA's numerous and increasing applications across the cleaning and personal care space, PVA is referred to as one of the most ubiquitous pollutants in waterways and soil.

PVA has various potential impacts on the soil and waterways it pollutes. Further research is still needed to determine the potential harms of PVA in the environment; however, PVA could have the following impacts:

PVA in soil: PVA that is released from WWTPs as sludge often ends up on land and in soil. This has the potential to affect agricultural yields.³⁴

PVA in aquatic environments: A study shows that PVA can alter gas exchanges within aquatic environments. In addition, PVA has exhibited bioaccumulation properties; it has

³³ Oceans, Plastic. "Detergent Pods Contributing to Plastic Pollution." *Plastic Oceans International* (blog), July 23, 2021. <https://plasticoceans.org/detergent-pods-contributing-to-plastic-pollution/>.

³⁴ *Ibid.* 12

“even been documented to mobilize heavy metals from sediments to water resources. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater. Some of these are proven carcinogens with great aqueous phase stability. As the sorption of organic and inorganic pollutants is not limited to hydrophobic compounds but can also occur with hydrophilic compounds, PVA could act as a vector for transport up the food chain, similarly to more conventional plastics.”³⁵

Section 5: Requested Actions:

Request for Health and Environmental Safety Tests under the Toxic Substances Control Act

Given the potential for PVA to persist in the environment as a harmful plastic pollutant, this petition requests that the EPA require health and environmental safety tests under the Toxic Substances Control Act on PVA and ultimately regulate PVA as a toxic substance, pending the results from testing. The Toxic Substances Control Act seeks to protect human health and the environment by empowering the EPA to issue testing requirements for specific chemicals and establish regulations that restrict manufacturing, processing and distribution of chemicals that are determined a health or environmental risk.³⁶

The Toxic Substances Control Act from 15 US Code Chapter 53 Subchapter I section 2603 states:

“If the Administrator finds that—

(A)(i) (I)the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment,

(II)there is insufficient information and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(III)testing of such substance or mixture with respect to such effects is necessary to develop such information; or

(ii)(I)a chemical substance or mixture is or will be produced in substantial quantities, and (aa) it enters or may reasonably be anticipated to enter the environment in

³⁵ *Ibid.* 12

³⁶US EPA, “Toxic Substances Control Act (TSCA) and Federal Facilities.”

substantial quantities or (bb) there is or may be significant or substantial human exposure to such substance or mixture,

(II)there is insufficient information and experience upon which the effects of the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(III)testing of such substance or mixture with respect to such effects is necessary to develop such information; and

(B) in the case of a mixture, the effects which the mixture's manufacture, distribution in commerce, processing, use, or disposal or any combination of such activities may have on health or the environment may not be reasonably and more efficiently determined or predicted by testing the chemical substances which comprise the mixture;

the Administrator shall by rule, or, in the case of a chemical substance or mixture described in subparagraph (A)(i), by rule, order, or consent agreement, require that testing be conducted on such substance or mixture to develop information with respect to the health and environmental effects for which there is an insufficiency of information and experience and which is relevant to a determination that the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture, or that any combination of such activities, does or does not present an unreasonable risk of injury to health or the environment.³⁷

Due to the unknown dangers that PVA poses to the environment, the EPA has an obligation, under the TSCA, to test PVA and its end of life in marine and aquatic ecosystems, as well as soil environments, to determine the implications for human and environmental health. This petition requests that the EPA complete full environmental and human health tests on both untreated and treated PVA that is released into aquatic, marine and land environments.

Request to Remove PVA from EPA Safer Choice Program and Safer Chemical List

In addition to requesting that PVA be tested and ultimately regulated under the TSCA, this petition requests that PVA be removed from the EPA Safer Choice List and Safer Chemical Lists until the requested testing is complete.

The EPA Safer Choice Program has the following guidance on polymers:

“To be acceptable for labeled products, polymers must have low-concern characteristics.¹ Also, the requirements of this section apply to the low molecular weight components of

³⁷15 U.S.C. 53 §2603. Testing of chemical substances and mixtures

polymers (typically less than 1,000 daltons). Safer Choice encourages the use of degradable polymers whenever possible; only those that do not degrade into CMRs or PBTs (Persistence, Bioaccumulation, Toxins) will be allowed.”³⁸

If a polymer does break down into PBTs, it should be excluded from the EPA Safer Choice list. Using the definitions that the Safer Choice programs outline for persistence and bioaccumulation, PVA should be excluded from the EPA Safer Choice list.

1. Persistence: “The length of time the chemical can exist in the environment before being destroyed (i.e., transformed) by natural processes”.³⁹

Rolsky and Kelkar’s paper demonstrates that there is significant potential for PVA to persist within waterways, oceans and soils after it leaves WWTPs, with ~75% remaining intact post water treatment.⁴⁰

2. Bioaccumulation: “is a process in which a chemical substance is absorbed in an organism by all routes of exposure as occurs in the natural environment, e.g., dietary and ambient environment sources. Bioaccumulation is the net result of competing processes of chemical uptake into the organism at the respiratory surface and from the diet and chemical elimination from the organism including respiratory exchange, fecal egestion, metabolic biotransformation of the parent compound and growth dilution”.⁴¹

Rolsky and Kelkar cite Chiellini et. al to suggest that PVA has the potential to exhibit bioaccumulation properties, citing its ability to “mobilize heavy metals from sediments to water resources. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater”.⁴²

PVA clearly demonstrates the EPA definitions of Persistence and has the potential for Bioaccumulation. Partially degraded PVA might have the potential to interact with or carry PBT chemicals and bioaccumulate them up the food chain. Because of these characteristics, PVA should not be eligible for the polymer exclusion and should be removed from the EPA Safer Choice List unless it can be proven to be safe.

³⁸ “EPA’s Safer Choice Criteria for Colorants, Polymers, Preservatives, and Related Chemicals,” n.d., 3.

³⁹ EPA PBT Final Rule [9]

⁴⁰ Rolsky and Kelkar, “Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate.” 12

⁴¹ EPA’s Safer Choice Program Master Criteria for Safer Ingredients

⁴² Chiellini, Emo, Andrea Corti, Salvatore D’Antone, and Roberto Solaro. “Biodegradation of Poly (Vinyl Alcohol) Based Materials.” *Progress in Polymer Science* 28, no. 6 (June 1, 2003): 963–1014. [https://doi.org/10.1016/S0079-6700\(02\)00149-1](https://doi.org/10.1016/S0079-6700(02)00149-1).

Lastly, PVA is used in all laundry and dishwasher pods and sheets. Many brands who use PVA market their pods and sheets as “100% biodegradable” and or “100% plastic-free”. Both claims are misleading, given that they use PVA, which is a petroleum-based plastic, and is only biodegradable under extremely specific conditions, as the research by Rolsky and Kelkar explains. These can mislead consumers to think these products are better for the environment than they are, when there is still further research needed. Even with further research, claims such as “100% biodegradable” and “100% plastic-free” would not be substantiated. We request that the EPA Safer Choice Program review claims about PVA through the lens of truth in advertising to ensure that consumers have accurate information about PVA and its potential environmental impacts.

Section 6: Conclusion

PVA plastic film is widely used in conventional household and personal care products. It is designed to become a solution when exposed to water before it is flushed down the drain. PVA can be biodegradable in WWTPs when it encounters a set of extremely specific conditions, as cited above. Unfortunately, as Rolsky and Kelkar found, these conditions are rarely met in the U.S. Instead, ~75% of PVA from laundry and dishwasher pods likely persists in waterways, soil and oceans.⁴³ Further research is needed to determine the potential hazards that polluted PVA can pose to ecosystems and human health. Given the gravity of the plastic pollution problem and the known impacts of other plastic polymers on the environment, this petition requests the EPA require extensive health and environmental safety testing of PVA once it is released into ecosystems by way of WWTPs, to determine potential environmental impacts. In addition, given the persistence of PVA in the environment and its potential for bioaccumulation, this petition requests immediate removal of PVA from the EPA Safer Choice List and EPA Safer Chemical Lists until required testing is conducted. Using the polymer exemption definition, the EPA puts forth, a polymer cannot be exempt if it degrades into PBTs (Persistence, Bioaccumulation, Toxins).⁴⁴ Rolsky and Kelkar model that PVA likely persists in our environment and Chiellini et al. have shown the potential for PVA to have bioaccumulation properties.⁴⁵ For these reasons, it is requested that PVA undergo the requisite testing to determine its impact on the environment and be removed from the Safer Choice Program until the tests are complete.

⁴³ Rolsky and Kelkar, “Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate.” 1.

⁴⁴ EPA PBT Final Rule [9]

⁴⁵ Chiellini et al., “Biodegradation of Poly (Vinyl Alcohol) Based Materials.”

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<http://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim>.
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EXECUTIVE DOCUMENTS

FEDERAL COMPLIANCE WITH POLLUTION CONTROL STANDARDS

For provisions relating to the responsibility of the head of each Executive agency for compliance with applicable pollution control standards, see Ex. Ord. No. 12088, Oct. 13, 1978, 43 F.R. 47707, set out as a note under section 4321 of Title 42, The Public Health and Welfare.

§2602. Definitions

As used in this chapter:

(1) the ¹ term "Administrator" means the Administrator of the Environmental Protection Agency.

(2)(A) Except as provided in subparagraph (B), the term "chemical substance" means any organic or inorganic substance of a particular molecular identity, including—

- (i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and
- (ii) any element or uncombined radical.

(B) Such term does not include—

- (i) any mixture,
- (ii) any pesticide (as defined in the Federal Insecticide, Fungicide, and Rodenticide Act [7 U.S.C. 136 et seq.]) when manufactured, processed, or distributed in commerce for use as a pesticide,
- (iii) tobacco or any tobacco product,
- (iv) any source material, special nuclear material, or byproduct material (as such terms are defined in the Atomic Energy Act of 1954 [42 U.S.C. 2011 et seq.] and regulations issued under such Act),
- (v) any article the sale of which is subject to the tax imposed by section 4181 of the Internal Revenue Code of 1986 [26 U.S.C. 4181] (determined without regard to any exemptions from such tax provided by section 4182 or 4221 or any other provision of such Code) and any component of such an article (limited to shot shells, cartridges, and components of shot shells and cartridges), and
- (vi) any food, food additive, drug, cosmetic, or device (as such terms are defined in section 201 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321]) when manufactured, processed, or distributed in commerce for use as a food, food additive, drug, cosmetic, or device.

The term "food" as used in clause (vi) of this subparagraph includes poultry and poultry products (as defined in sections 4(e) and 4(f) of the Poultry Products Inspection Act [21 U.S.C. 453(e) and (f)]), meat and meat food products (as defined in section 1(j) of the Federal Meat Inspection Act [21 U.S.C. 601(j)]), and eggs and egg products (as defined in section 4 of the Egg Products Inspection Act [21 U.S.C. 1033]).



Communication

Degradation of Polyvinyl Alcohol in US Wastewater Treatment Plants and Subsequent Nationwide Emission Estimate

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Abstract: Polyvinyl alcohol (PVA) is a water-soluble plastic commercially used in laundry and dish detergent pods (LDPs) for which a complete understanding of its fate in the environment and subsequent consequences is lacking. The objective of this study was to estimate the US nationwide emissions of PVA resulting from domestic use of LDPs, corroborated by a nationwide, online consumer survey and a literature review of its fate within conventional wastewater treatment plants (WWTPs). Peer-reviewed publications focusing on the degradation of PVA in critical processes of WWTPs were shortlisted as a part of the literature review, and subsequent degradation data was extracted and applied to a model with a set of assumptions. Survey and model results estimated that approximately $17,200 \pm 5000$ metric ton units per year (mtu/yr) of PVA are used from LDPs in the US, with $10,500 \pm 3000$ mtu/yr reaching WWTPs. Literature review data, when incorporated into our model, resulted in ~61% of PVA ending up in the environment via the sludge route and ~15.7% via the aqueous phase. PVA presence in the environment, regardless of its matrix, is a threat to the ecosystem due to the potential mobilization of heavy metals and other hydrophilic contaminants.

Keywords: biodegradation; dish detergent; laundry; mass loads; microbes; polyvinyl alcohol; wastewater



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1. Introduction

Plastic pollution has been steadily increasing since the 1950s [1]. Due to an upsurge in public awareness regarding plastic usage and pollution, more “sustainable” alternatives have increased in popularity, and are thus being utilized in higher quantities by the general public [2]. These new materials are often marketed as “biodegradable”, as they are considered to be susceptible to microbial degradation under specific conditions, but this specificity often makes it difficult to understand their ultimate fate in the environment. Polyvinyl alcohol and its corresponding blends (PVOH, PVAI, Polyviol, Alcotex, Covol, Gelvatol, Lemol, Mowiol, Mowiflex, and Rhodoviol) are examples of polymers that have become more popular both in usage and within scientific research (see Figure 1A) due to their water-solubility. Typically, PVA is used as a protective film for laundry and dish detergents; as a sizing and finishing agent in the textile industry [3]; and as a thickening or coating agent for paints, glues, meat packaging, and pharmaceuticals in paper and food industries (see Figure 1A) [3].

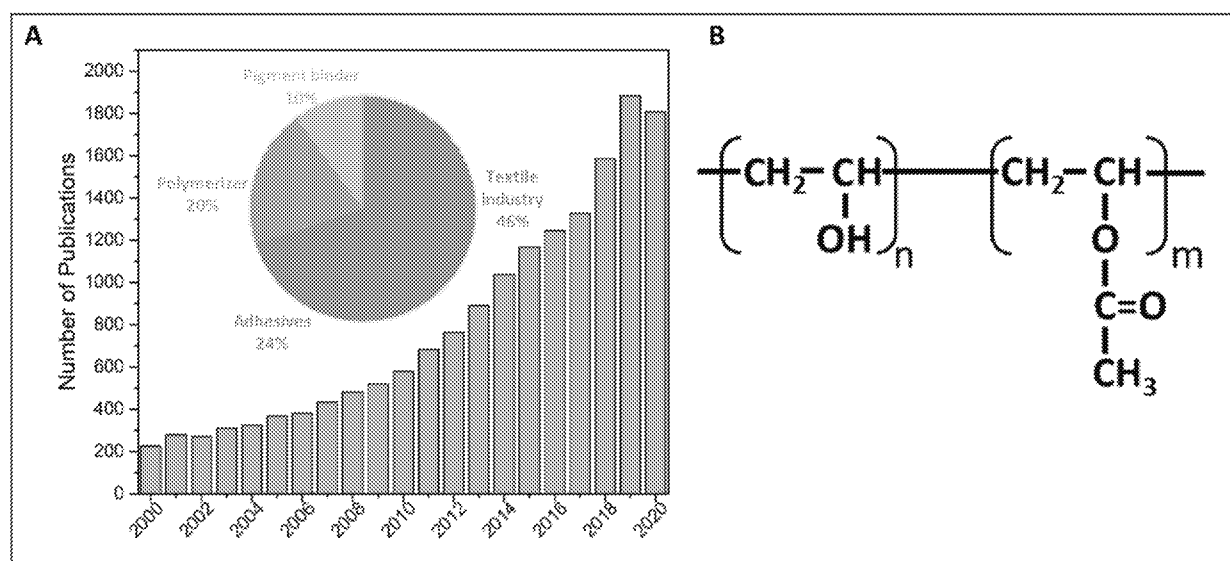


Figure 1. (A) Increasing number of publications per year focusing on polyvinyl alcohol (PVA) as well as a pie chart depicting percentage distribution of PVA applications [3] and (B) the chemical structure for partially hydrolyzed polyvinyl alcohol-acetate.

Up to 650,000 tons of PVA is produced yearly across the globe [4] and this has been expected to increase 4.09% annually from 2018 to 2023 [5]. In 2018, due to its general increase in usage, PVA was considered to be one of the most ubiquitous pollutants in wastewater [4,6,7]. A thorough understanding of its path to and breakdown within the environment is presently lacking. Although water-soluble, its constituents, such as ethylene (a petroleum-based product), can remain intact within the solvent. Studies have shown ethylene to have negative effects on surrounding organisms, such as plants, which naturally produce and utilize ethylene [8]. Similar to table salt and sugar, PVA dissolves in water, and if the water volume is low, a viscous solution will be formed. The high water volume in WWTPs means the texture of the water should remain unchanged. When PVA is discharged into water bodies, it has the ability to foam due to its surface properties [9]. This can inhibit oxygen transfer, causing irreparable harm to aquatic life [10]. Additionally, because of its hydrophilicity, PVA has the potential to adsorb dangerous chemicals or contaminants [11], such as antibiotics [12] or heavy metals [13–15], at high concentrations. These can then concentrate up food chains [16], posing a threat to the environment, similar to behavior of traditional polluted plastics. WWTPs are known to contain a variety of dangerous contaminants, creating a higher-risk situation for PVA particles passing through [17].

The PVA used in LDPs is composed of PVA polymeric chains with a fraction of polymeric acetate groups (see Figure 1B). This is referred to as partially hydrolyzed PVA, and the percentage of hydrolysis and molecular weights vary with its application [18,19]. The PVA used in LDP blends is typically 88% hydrolyzed [18], while its molecular weight can vary within several ranges, including but not limited to 1000–1,000,000, 10,000–300,000, and 20,000–150,000 Da [19]. Upon contact with water, the presence of polymeric acetate groups enables the water molecules to penetrate the bonds of the outer coating and break it into smaller chains. Once flushed down the drain, partially hydrolyzed PVA chains (See Figure 1B) enter wastewater channels, eventually interacting with WWTPs. The fate of PVA in wastewater treatment systems has been partially explored, with some studies highlighting specific aspects of the wastewater treatment process, but very few studies aimed to establish a complete degradation estimate in conventional WWTP processes from beginning to end.

Available studies suggest that the degradation of PVA occurs under a specific set of circumstances, which may not be ubiquitous within WWTPs or the natural environment. Ultimately, PVA degradation is reported to be a slow process, which greatly depends upon

the surrounding conditions mentioned in Table S2 [20]. Bacteria utilize enzymes to degrade PVA to its constituent form by attacking specific bonds within the polymeric chain [7,21]. Bacteria oxidizes the tertiary carbon atoms, leading to the endo-cleavage of PVA molecules, one of the main degradation routes, which leads to the creation of byproducts such as hydrolyzable hydroxy ketone and 1,3-diketone [20,22]. Other microorganisms mainly utilize PVA as a carbon source; such is the case with the bacteria *Pseudomonas* [20], which generate hydrogen peroxide and other byproducts, including a lower molecular weight PVA [23,24]. Many of these processes can take place simultaneously to begin the degradation of the polymer [20]. While several bacterial species have been documented degrading PVA, these are infrequently found within conventional WWTPs or the environment [20], as are the other optimal circumstances necessary for PVA to completely degrade (Table S1). WWTPs are predominantly designed to remove suspended solids, harmful bacteria, and pollutants of emerging concern [25], but the removal of PVA is still in question due to a lack of comprehensive research. Independent studies have been performed demonstrating removal of PVA using different bacteria, enzymes, and chemical processes [20], but a complete fate assessment in a conventional WWTP or the environment is currently lacking.

In this study, we present a detailed qualitative, quantitative, and spatial analysis on annual mass emissions of PVA in the US, consisting of three defined segments: (i) an online survey involving 500+ respondents investigating their laundry and dish detergent purchasing habits (ii) a United States Geological Survey (USGS) report on water usage, sources, and population; (iii) and a detailed literature review on the degradation of PVA in wastewater, utilizing the data to conduct a subsequent mass balance analysis.

2. Materials and Methods

2.1. Online Survey

An online survey was conducted with 527 respondents, 80% female and 20% male, ages 24–55 years. The survey was directed at the primary decision-maker responsible for purchasing cleaning products in the household. In total, 60% of the responses were from the top 20 designated market areas (DMAs). Survey questions mainly investigated the frequency of buying laundry and dish detergent and the type of detergent bought (single dose pods or detergent bottle) (see Supplemental Information).

2.2. Water Use and Wastewater Generation in the US

State wise data on water use and application for the United States was published in the USGS report titled “Estimated Use of Water in the United States in 2015” [26]. Treatment plants in the US receive wastewater from public facilities, domestic sources, and industrial effluents. Hence, the total water used that ultimately resulted in the generation of wastewater was the sum of public, domestic, and industrial supply (Mgal/d) as shown in Equation (1) which assisted in populating Figure 2.

$$W_{Use,2015} = W_P + W_D + W_I \quad (1)$$

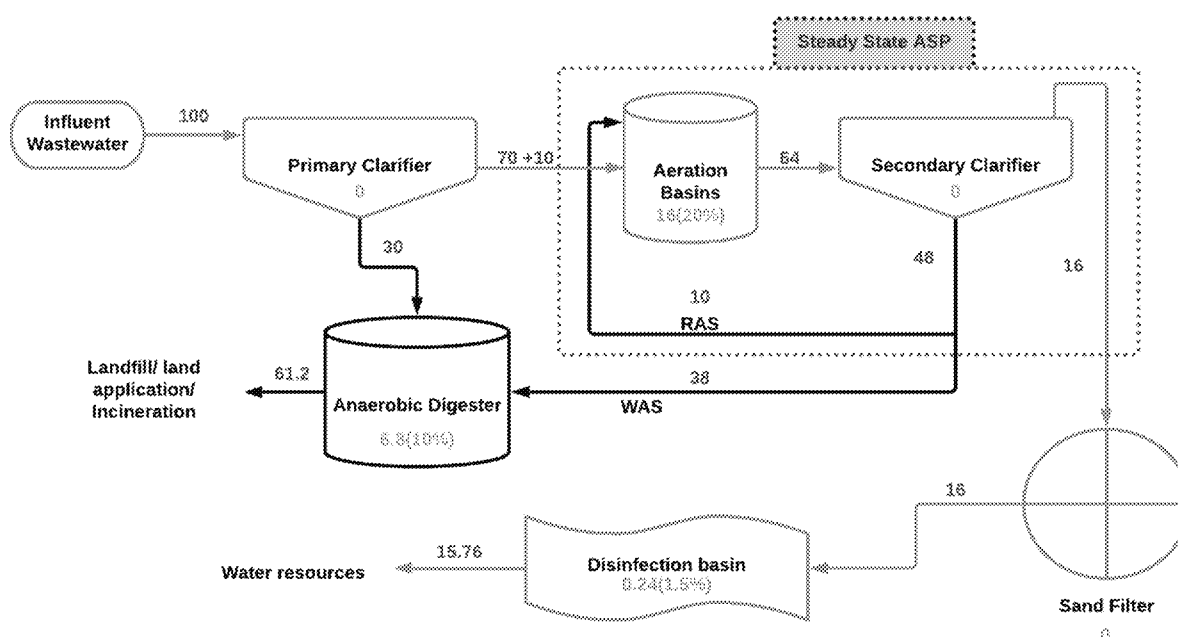


Figure 2. Mass balance of PVA in a conventional activated sludge treatment plant, considering clarifier efficiencies and biodegradation efficiencies. Numbers in red indicate the percentage of PVA in respective treatment streams, and numbers in green represent the amount (% absolute) of degraded PVA in respective sections. RAS and WAS represent return activated sludge and waste activated sludge, respectively. Numbers in parentheses represent the degradation efficiencies of respective sections.

In this equation, W_P , W_D , and W_I stand for public, domestic, and industrial water supplies, respectively.

Not all water used is received by WWTPs as wastewater; there are an estimated 20%–25% in losses [27], which include but are not limited to leakages of sewers and sanitary sewage outflows.

The total wastewater generated was calculated using Equation (2), assuming a 20% loss in volume. In this case, $WW_{G,2015}$ stands for the wastewater generated for each state.

$$WW_{G,2015} = 0.8 * (W_P + W_D + W_I) \quad (2)$$

Roughly 16,000 US WWTPs treat 34 billion gallons (BGD) of wastewater daily [28]. This represents an average of approximately 2.12 billion gallons treated daily per facility. Wastewater treated for each state can be calculated using Equation (3), where N_F and $WW_{T,2015}$ represent the number of wastewater treatment facilities in each state and amount of wastewater treated, respectively, assuming a complete use of operational treatment capacity.

$$WW_{T,2015} = N_F * 2.12 \text{ BGD} \quad (3)$$

The untreated wastewater was calculated as the difference between generated and treated wastewater, as shown in Equation (4). Untreated wastewater could be a result of several possibilities, including but not limited to the lack of connectivity between households and WWTPs, improper disposal of wastewater generated, and/or generated wastewater exceeding the operational capacity of a WWTP (current assumption).

$$WW_{UT,2015} = WW_{G,2015} - WW_{T,2015} \quad (4)$$

Subsequently, the percentage of wastewater untreated and treated were calculated as shown in Equations (5) and (6) respectively.

$$WW_{UT,\%} = \frac{WW_{UT,2015}}{WW_{G,2015}} * 100 \quad (5)$$

$$WW_{T,\%} = \frac{WW_{T,2015}}{WW_{G,2015}} * 100 \quad (6)$$

2.3. LDP Usage and Treatment

Laundry pods from three brands and dish pods from two brands were drained, air-dried overnight, and weighed on an Ohaus Adventurer weighing scale (AR1530, China). Laundry pods from different brands ($n = 9$) and dish pods from varying brands ($n = 6$) were weighed in triplicate. The average weights for laundry ($M_{L,Avg}$) and dish ($M_{D,Avg}$) pods were 1.0 ± 0.6 g and 0.5 ± 0.2 g, respectively.

Data from the online survey revealed consumption of ~15 billion laundry pods ($N_{L,Avg}$) and ~12 billion dish pods ($N_{D,Avg}$) per year by 126 million households in the US. Using 2015 state-wise population numbers from the USGS report [29] (P_{2015}), the number of per capita pods consumed in the US ($N_{PC,Avg}$) was calculated using Equation (7).

$$N_{PC,Avg} = \frac{N_{L,Avg} + N_{D,Avg}}{P_{2015}} \quad (7)$$

The outer dissolvable coating is composed of PVA and other additives in varying proportions. Based on patents and reports, the PVA ratio (by weight) lies between 65% and 99% of the total outer coating weight [30]. An average mass of PVA in laundry and dish pods was calculated using Equation (8), where f_L and f_D are the fractions of PVA in laundry and dish pods, respectively, in grams.

$$M_{PVA,Avg} = M_{L,Avg} * f_L + M_{D,Avg} * f_D \quad (8)$$

The number of pods (laundry and dish) used by each state was calculated as per Equation (9).

$$N_{Pod} = N_{PC,Avg} * P_{2015} \quad (9)$$

The mass of discarded PVA from laundry pods ($M_{L,G}$) and dish pods ($M_{D,G}$), in each state was expressed as per Equation (10).

$$M_{PVA,G} = N_{Pod} * M_{PVA,Avg} \quad (10)$$

Untreated and treated masses of PVA were calculated as per Equations (11) and (12), respectively.

$$M_{UT,PVA} = M_{PVA,G} * WW_{UT,\%} \quad (11)$$

$$M_{T,PVA} = M_{PVA,G} * WW_{T,\%} \quad (12)$$

Applying total degradation percentages (solid + aqueous phase) from the modeled scenario to the PVA treated ($M_{T,PVA}$) would result in total PVA emissions from WWTPs.

2.4. GIS and Mapping

Figure 3 was created in Arc GIS pro 2.0.0. Data specific to the US states and environmental emissions of PVA (metric tons/yr) were collected from outside sources. These were then imported into the GIS software program ArcMap.

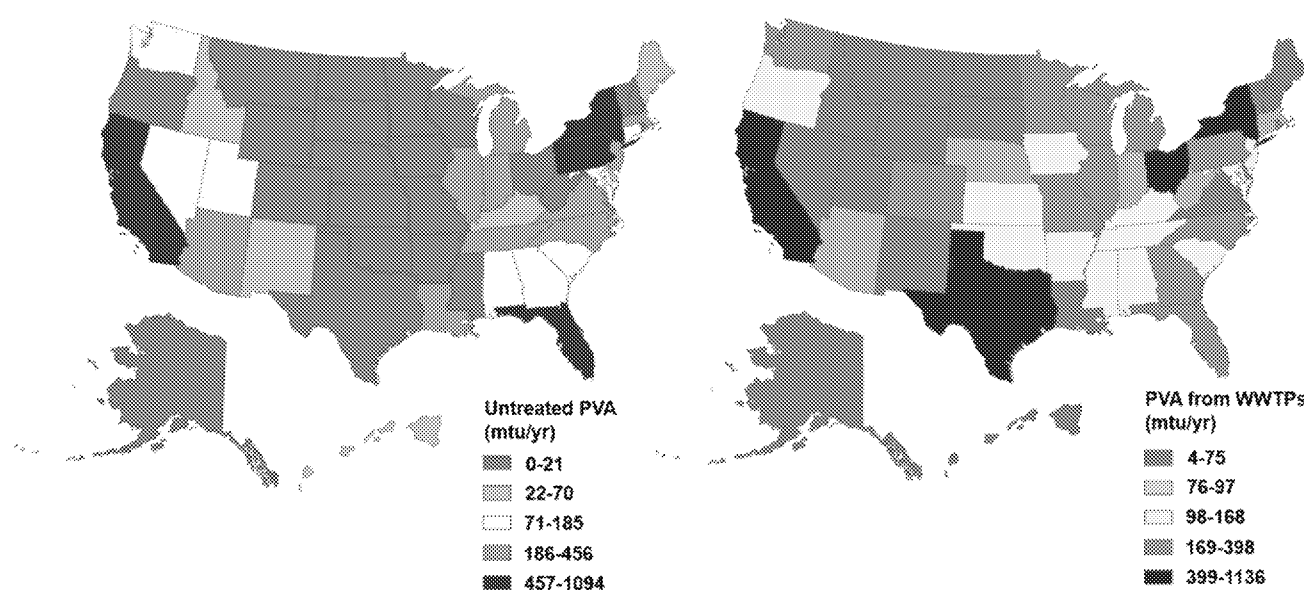


Figure 3. PVA emissions across the U.S. in mtu/yr. The left panel is the spatial distribution of untreated PVA via wastewater that does not reach the treatment plants. The right panel represents the PVA from WWTP effluent streams, including aqueous and sludge disposal routes.

2.5. Literature Review

A comprehensive literature review was performed on the presence and degradation of PVA in US wastewater according to PRISMA guidelines. A detailed breakdown of the publication selection is presented in SI Figure S1. Databases accessed included Google Scholar and Scopus, utilizing the search terms polyvinyl alcohol*; PVA*; Polyvinyl alcohol with results further specified using the constraining term (AND): Pollution*; Degradation*; Biodegradation*; Wastewater*; WWTP*; Sludge*; Sewage*; Effluent*; Influent*; Activated sludge*; US. Papers published between 1950 and 2020 were sought out. These studies were specifically sought out as they aimed to assess the breakdown of PVA within a conventional WWTP by microbial attack or other forces, such as UV radiation or chlorine oxidation. From each relevant paper, the degradation mechanism was extracted as well as the percent degradation, WWTP section (if applicable), the microorganism species, and whether or not the species was adapted to the wastewater itself. As many of the papers included several independent studies relating to PVA degradation in wastewater, a range of percentages were then included to encapsulate all reported values within a single manuscript. Papers with incomplete text or a lack of relevance to wastewater were excluded (see Figure S1).

3. Removal of PVA in WWTPs

The majority of the PVA, generally in the form of greywater from domestic, public, and industrial sources, reaches WWTPs, where it encounters primary treatment, secondary treatment, sludge treatment, and disinfection before leaving the WWTP.

3.1. Primary Treatment

Primary treatment consists of initial screening, grit removal, and a primary clarifier, with the objective of removing coarse solids and other large items [31]. PVA has not been studied in the context of its partitioning and removal in primary treatment within a conventional WWTP. Degrading PVA is a challenge and can contribute to the total chemical oxygen demand (COD) [32] from the incoming wastewater. The most influential mechanism of removal in a primary clarifier is the sorption to the suspended solid particles [33]. However, PVA is a hydrophilic polymer and has a greater affinity toward water, tending to stay in the liquid phase as opposed to solid [34]. It is possible that PVA could form gelatinous consistencies with fats and lipids from influent wastewater, which may increase its partition

toward solid matter and its removal from the aqueous phase. An empirical study analyzing hydrophilic pharmaceuticals and endocrine-disrupting chemicals in wastewater revealed the low sorption capacity of hydrophilic contaminants to solids, requiring relatively longer retention times (5–11 h) for their efficient removal from the aqueous phase [35]. Current literature evidence is not sufficient to establish a definitive path PVA may take in a primary treatment system, but the COD removal efficiency of the primary clarifiers likely eliminates PVA from the aqueous phase [36].

3.2. Secondary Treatment

Secondary treatment is designed to further remove organics and suspended solids [31]. Two main components of secondary treatment are activated sludge processing and the use of the secondary clarifier.

3.2.1. Activated Sludge Process (ASP)

The activated sludge process involves the recycling of the bacteria/microbes present in the sludge, which are sent back into the aeration chambers. These microbes are responsible for the degradation of organic waste and chemicals from the influent wastewater. The bulk of PVA degradation is anticipated to occur at this stage. There are several key factors influencing the biodegradation of PVA in an ASP. One important element is the food-to-microorganisms ratio (F:M), defined as the load of substrate applied daily per unit of biomass [37]. A general F:M ratio lies anywhere between 0.25 and 0.45 [38]. However, an F:M ratio better suited for PVA degradation lies between 0.1 and 0.15 [39]; thus, a higher number of microorganisms is required to fully degrade PVA, compared with conventional domestic sludge. Typical microorganisms may not be able to adequately break down PVA, as the presence of PVA-adapted microbes is necessary for thorough biodegradation of PVA in the ASP [22,39,40]. PVA adaption often requires a lag time spanning over several weeks [22,39], and the ASP can only be PVA-adapted in WWTPs receiving a heavy influx from textile industries, allowing sufficient time for the microbes present at the facility to adapt to the incoming COD. After the lag phase is complete, biodegradation thereafter occurs exponentially [39].

The number of PVA-degrading microbes is limited, and their presence is specific to certain environments and environmental conditions. Schonberger et al. discovered that WWTPs consistent with PVA adaption within the ASP achieved 80% degradation in a 7-day period. Alternatively, the unadapted ASP was consistent with only 18% biodegradation within the same time frame [39]. Hoffman et al. observed similar findings when four different blends of PVA were tested in adapted and unadapted sludge. In unadapted sludge, a degradation of $88 \pm 9\%$ of incoming PVA was achieved in 187 ± 25 h for four blends, and $\sim 20\%$ was degraded in 25 h [22]. For adapted sludge, $90 \pm 5\%$ was degraded in just 29 ± 2 h [22]. The above findings utilized 88% hydrolyzed PVA, the same composition as that used in detergent pod coatings [30,41].

The average hydraulic retention time (HRT) in the ASP is approximately 18–24 h, and the sludge retention time (SRT) is 12–15 days [42]. Due to the hydrophilic nature of PVA, the majority of PVA is expected to be in the water phase, in which the HRT would subsequently play a larger role in its degradation.

3.2.2. Secondary Clarifier

The decomposed sludge mixture then enters the secondary clarifier, where the solid waste is given time to settle, allowing the liquid to enter the tertiary treatment stage. In the secondary clarifier, three removal mechanisms may occur, which include volatilization, biodegradation, and adsorption to solids [33]. The current literature does not present evidence of volatilization, biodegradation, or adsorption of PVA in secondary clarifiers. However, since the majority of secondary sludge contains fluid, and the density of PVA is ~ 1.2 g/cm³, a fraction of PVA may settle and exit via secondary sludge.

3.3. Tertiary Treatment

Tertiary treatment typically consists of a disinfection chamber and a filtration unit. Older WWTPs use chlorination as an effective disinfectant, along with trickling or gravity filters, due to their moderate efficiency and low cost [43]. These technologies are evolving, as most modern WWTPs rely on advanced oxidation processes (AOPs) and membrane filtration, according to the United States Environmental Protection Agency (USEPA) [44].

3.4. Disinfection

In 2017, Ye et al. studied the comparative effects of using UV, chlorine, and a UV-chlorine combination in PVA degradation/oxidation [45]. Their experiments revealed that 20 min of UV irradiation ($I_s = 2.6 \text{ mW/cm}^2$) had a minimal effect on the initial concentration of PVA (50 mg/L). Chlorine had a similar result, with just 1.5% of original PVA degraded in 20 min. Chlorine conditions of 20 mg Cl_2/L and a pH of 7 are synchronous with WWTP disinfection conditions in the US [46]. However, a combination of UV-chlorine treatment resulted in 92% degradation within 10 min and 100% in 20 min. Advanced oxidation technologies have been documented to eliminate PVA completely from wastewater in a matter of minutes [15]. These processes can be expensive and thus not cost-effective for the municipality.

In 2004, the US EPA reported that chlorination is the most utilized method for wastewater disinfection (EPA primer, 2014). Given the degradation conditions under chlorine alone, only a minimal amount of PVA coming from domestic wastewaters can be expected to be degraded.

3.5. Filtration

Sand filtration is one of the most widely used filtration techniques due to its low cost of operation and maintenance [47]. Research on the behavior of PVA in a sand or trickling filter system is lacking. However, in cases of missing data, its fate can be predicted based on the behavior of other commonly used hydrophilic materials, such as pharmaceuticals with an octanol-water partitioning coefficient (log Kow) below 2 (see Table 1). A low log Kow indicates the compound's affinity toward water and categorizes the compound as hydrophilic in nature. The table below demonstrates the very low removal efficiencies of widely known hydrophilic pharmaceuticals in a sand filtration system. It should be noted that sand filtration had minimal to no effect on the concentrations of the compounds studied, with a mean and standard deviation of $-0.08\% \pm 20\%$. It is likely that sand filtration would not have a significant effect on PVA concentrations.

Table 1. Removal efficiencies of hydrophilic pharmaceuticals and their log Kow factors in filtration systems.

Compound	RE (%)	log Kow
Ibuprofen	21 [48]	2.48
Gemfibrozil	17 [48]	4.77
Diclofenac	9 [48]	1
Fenofibric acid	5 [48]	1.9
Clofibric acid	15 [48]	2.88
Carbamazepine	1.4 [49]	1.51
Doxycycline	−13 [49]	−0.62
Oxytetracycline	−33 [49]	−0.9
Sulfadiazine	7.7 [49]	−0.09
Acetaminophen	−40 [49]	0.46

3.6. Anaerobic Digestors

Wastewater activated sludge coming from secondary clarifier effluent is treated in an anaerobic digester to reduce the overall volume, destroy pathogens, and control odors [50]. Using anaerobic microbes, the organic matter is further broken down, releasing methane

and water as byproducts. This treated sludge, termed “biosolids”, is then safe to be used for agricultural purposes, as an example. In the US, anaerobic digesters are typically catered towards mesophilic (30–37 °C) and thermophilic (50–60 °C) bacteria with retention times of 12–25 and 10–12 days, respectively [50].

Matsumura et al. explored PVA degradability using a lab-scale anaerobic digester with activated sludge obtained from a local WWTP in Japan [51]. Two drastically different molecular weights (14,000 and 2000 Da) were selected for the study. In the first 25 days, both PVA blends showed similar biodegradation results of 12.5%. As time progressed, PVA-14,000 degraded at a much higher rate compared with PVA-2000. After 150 days, PVA-14,000 reached 50% degradation, while PVA-2000 was only 37% degraded [51]. Such low biodegradation rates could be attributed to the usage of PVA-unadapted sludge.

Different PVA-starch blends were studied by Russo et al. under anaerobic conditions. They studied starch to PVA ratios of 90:10, 75:25, 50:50, and 0:100. Anaerobic conditions of 38 ± 5 °C with microbes sourced from activated sludge processes were maintained under nitrogen for up to 100 h. Because PVA used in detergent pods does not contain any starch additives [30], the results of the starch to PVA blend of 0:100 most closely mimicked detergent-derived PVA behavior. The solubilization study yielded lower results for the 0:100 blend, with only 10% of the PVA able to solubilize under anaerobic conditions in 100 h. Its 90:10 counterpart was solubilized up to 60% in the same time allotment [52]. Anaerobic digestion efficacy is often assessed based on the amount of methane and carbon dioxide produced as a byproduct. Upon isolating methane and CO₂ production analysis, the 0:100 blend produced less than 5 mL/g COD, whereas the 90:10 blend produced ~40 mL/g COD [52]. These results highlight the inefficiency of traditional anaerobic digestion methods for the biodegradation of PVA.

Another study analyzing the breakdown of PVA-glycerol-starch blends in anaerobic digestors was conducted by Pšejka et al. in 2006 [53]. After 30 days of incubating PVA blends at 35 ± 2 °C with anaerobic bacterial cultures, the percentage of biodegradation was assessed based on the balance of carbon, biogas, as well as the liquid phase [53]. The bacterial inoculum was sourced from unadapted municipal sludge of a local WWTP. A total of 13 blends were studied, including 85%/15% PVA-glycerol, 70%/15% PVA-starch, and a 75%/15%/10% PVA/starch/glycerol blend. The biodegradation percentages varied from 4.1% to 19.8%. The 85%/15% PVA-glycerol blend degraded the least, at 4.1% in 30 days, whereas the 75%/15%/10% PVA/starch/glycerol blend degraded the most, at 19.8% [53]. According to the authors, high degradation rates (high carbon differential) can be attributed to the biodegradation of starch, not the PVA itself. Since detergent pods do not contain starch additives, high biodegradation rates may not be observed during anaerobic digestion.

4. Estimated Mass Balance

The mass balance presented here is a combination of degradation percentages adopted from Sections 3.1–3.6, as well as a WWTP scenario assumed by Garrido et al. in 2013 [54]. Additionally, WWTP assumptions similar to those of Garrido et al. regarding primary and secondary clarifier COD removal efficiencies were also adopted. A variety of scenarios can be suggested based on a high number of variations in treatments across the US. However, many US WWTPs are older in age and rely on conventional treatment technologies. A conventional activated sludge facility (completely mixed) with primary treatment (screening, grit removal, and primary clarifier), secondary treatment (aeration basins, secondary clarifier, and activated sludge process), tertiary treatment (disinfection and sand filtration), and anaerobic digester was assumed for this study. The COD removal efficiency of the primary clarifier was fixed at 30% and 75% for the secondary clarifier [54]. PVA was considered to be a part of the total COD in this model, and the efficiencies signifying the percentage of the partitioning of COD within the clarifiers were directly applied to PVA. Table 2 indicates the treatment section and the corresponding degradation percentages for the SRT, HRT, and other process conditions based on the literature review within this study. PVA in the

influent wastewater entering primary treatment is considered to be 100%. As PVA passes the primary clarifier, 30% is expected to partition into the solid phase and eventually reach the anaerobic digester. The remaining PVA (70%) in the aqueous phase then enters the activated sludge system (see Table 2).

Table 2. The treatment section, corresponding degradation percentages, SRT, HRT, and other process conditions in a conventional sewage treatment plant. Other processes that do not contribute to degradation are excluded from this table.

Sr. No.	Process	HRT (h)	SRT (days)	Other Conditions	Degradation (%)
1	Activated sludge process	18–24 [42]	12–15 [42]	F:M ratio: 0.25–0.45 [38]	20
2	Anaerobic digestion	NA	25 [50]	PVA unadapted sludge 37 °C [50]	10
3	Disinfection	0.5 [46]	NA	PVA unadapted sludge chlorination 20 mgCl ₂ /L	1.5

NA: Not applicable.

The microbial activity in the aeration basins degrades 20% of the PVA in the presence of unadapted sludge during 18–24 h of HRT. Residual PVA (64%) enters the secondary clarifier, where 75% (48% of total) is partitioned into the sludge phase, and 25% (16% of total) is carried over via the liquid phase to the sand filters. The 48% in the sludge phase is further divided into 21% (10% of total) as return activated sludge (RAS) and 79% (38% of total) as waste activated sludge (WAS). Therefore, 10% of the total PVA is assumed to be in the form of return activated sludge (RAS). RAS values are adopted from the EPA report, in which RAS flow varied between 15% and 127% of the secondary influent flow, with the number eventually settling in the lower 20s [55]. RAS and primary effluent amount to 80% of the total PVA entering the aeration basin.

Solid phase effluent from the primary clarifier (30%) and WAS (38%) are treated in an anaerobic digester, where 10% is degraded (6.8% of the total) and 61.2% of the total is left untreated and ready to be landfilled, land applied, or incinerated.

The aqueous phase, containing 16% of the total PVA reaching the sand filtration stage, enters unaltered into the disinfection basin, where 1.5% is degraded (0.24% of the total) and 15.76% remains intact within the aqueous phase. A detailed mass balance can be found in Figure 2. References for degradation percentages and respective retention times are listed in Table 2.

5. Nationwide PVA Emissions via WWTPs (Effluent + Biosolids)

Referencing the above model, it is estimated that ~61.2% of PVA is emitted via sludge, and ~15.7% is emitted through effluent, with ~77% of the PVA still intact after passing through conventional wastewater treatment (see Figure 2). Once PVA has passed through conventional water treatment, either untreated or within sludge, its journey and fate become important to understand. By accessing data relevant to sewage treatment in US states, we were able to project the amount of intact PVA emitted by each US state. Our research indicates that the Midwest of the US has the lowest amount of untreated PVA, possibly due to lower populations and fewer population-dense areas (see Figure 3, left panel). The Southern and some Western areas of the US have lower to moderate volumes of PVA emissions. However, states such as California, Florida, New York, and Pennsylvania have the highest loadings via untreated wastewater. This may be due to the presence of more metropolitan cities and overworked public facilities as populations grow, causing urban expansion [56].

PVA emissions via WWTP effluent demonstrated slightly different data, with certain regions remaining the same (see Figure 3, right panel). Similar to untreated PVA emissions, much of the Midwest had lower emissions, with the South showing slightly higher numbers, and larger states with high treatment capacities, such as Texas, California, New York, and Florida, having the highest loadings via effluent. It is worth noting that most of

the states with the highest PVA emissions, either untreated or via effluent, have coasts bordering the Pacific or Atlantic Oceans, suggesting a quicker release of PVA into aquatic or marine ecosystems. Previous research has detected wastewater-derived contaminants in the ocean [57], and similar methods could be used to trace the presence of PVA within surrounding marine or terrestrial ecosystems. As human populations and their LDP usage continue to increase, it is expected that wastewater-derived contaminants will also increase [57].

Our data suggest that, on average, only $\sim 10,500 \pm 3000$ mtu/yr (See Figure 4) of PVA enters treatment infrastructure, and only a fraction of this is biodegraded due to the specificity of conditions required to facilitate complete degradation. Based on the assumed WWTP scenario, 15.76% remains in the aqueous phase ($\sim 1600 \pm 500$ mtu/yr) and 61.2% (6500 ± 1900 mtu/yr) remains in the biosolids exiting the anaerobic digester. Thus, a total of 8100 ± 2400 mtu/yr of PVA is estimated to remain untreated by WWTPs annually in the United States. Of that, 6500 ± 1900 mtu/yr of PVA remains untreated due to lack of treatment capacity or inaccessibility to a functioning WWTP in certain remote communities.

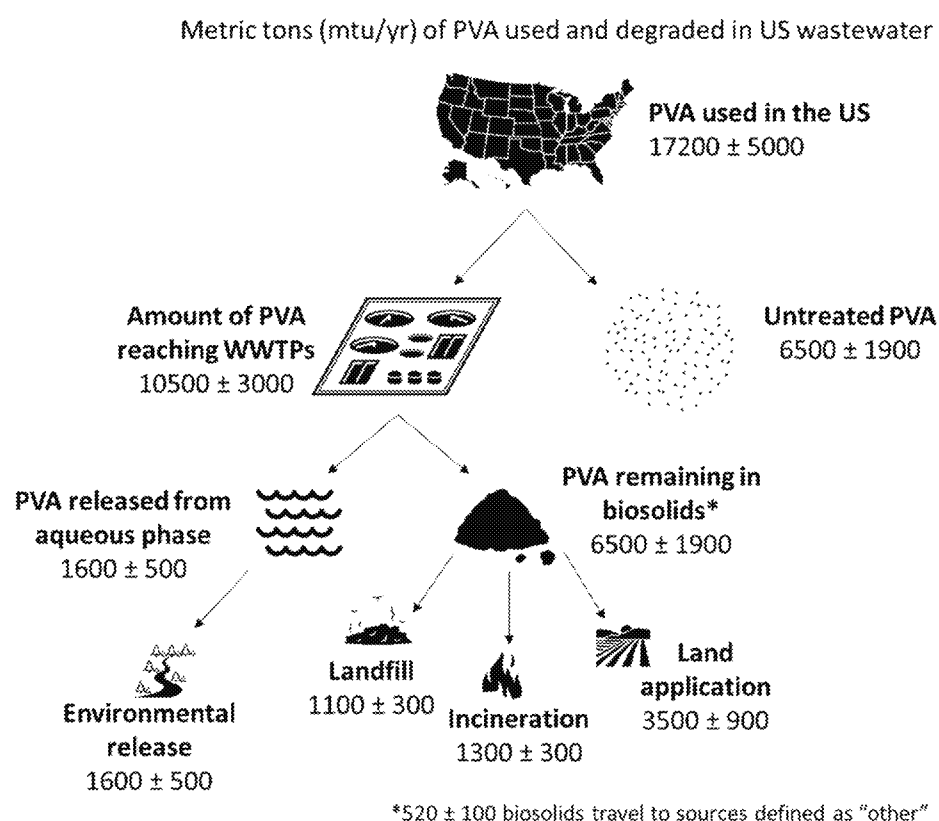


Figure 4. Modeled PVA usage and emissions in metric tons per year (mtu/yr) in the US.

Once biosolids leave a WWTP facility, 50–60% are applied to agricultural lands, 20% are sent to be incinerated, and 17% are sent to a landfill [58]. Each of these locations carries environmental risks associated with the distribution of plastics from biosolids. Initial research chronicling the negative impact of environmental PVA and future areas of study are listed in the following section.

6. Implications

The pathways of sludge have been well documented, as has the ability of WWTPs to act as sources of contaminants and microplastics entering the environment. These emissions can cause deleterious impacts on surrounding ecosystems and the biota within them. PVA that passes through conventional water treatment can similarly pose a threat to the

environment in several ways, once released into the environment or if land applied. Our data suggest that around 3500 mtu/yr of PVA is sequestered within agricultural soils in the US. As mentioned previously, ethylene is a byproduct of PVA degradation and is also a hormone utilized by plants. It is unknown if ethylene derived from PVA could affect agricultural yields, but it warrants investigation. The ability for plastic particles to adsorb dangerous contaminants at high concentrations has been documented, but this research is currently lacking as it pertains to PVA. Initial studies revealed that PVA can alter gas exchanges, such as carbon dioxide exchange, affecting aquatic ecosystems [20]. It is also capable of leaching into the groundwater, and it has even been documented to mobilize heavy metals from sediments to water resources [59–61]. Hydrophilic compounds, such as biocides, insecticides, herbicides, flame retardants, corrosion inhibitors, personal care products, and pharmaceuticals are present in wastewater and stormwater [62]. Some of these are proven carcinogens [63] with great aqueous phase stability. As the sorption of organic and inorganic pollutants is not limited to hydrophobic compounds but can also occur with hydrophilic compounds, PVA could act as a vector for transport up the food chain, similarly to more conventional plastics. During such phenomena, the contaminant concentrates, increasing its level of toxicity [61]. This area requires additional research in order to further elucidate the impact of intact PVA on the natural environment.

Prior research has demonstrated that WWTPs are sources of microplastic pollution in natural and built environments. This is due to the fact that microplastics in treated sludge, termed “biosolids”, can have a variety of harmful effects on ecosystems beyond contaminant adsorption. A sizable fraction of biosolids is deposited on agricultural soils as they serve as a rich form of fertilizer, ultimately improving soil properties [64]. If biosolids are contaminated with microplastics, the particles destabilize the benefits of sludge by negatively affecting microbial activity, bulk density, and water holding capacity of the soils [57]. A portion of biosolids is also sent to landfills around the US. It is thought that this process sequesters materials in the long term, but landfill leachate has been found to carry microplastics, prompting the consideration of landfills as a source of microplastics into the environment [65]. Lastly, a portion of US biosolids is incinerated. Research has shown that incineration does not terminate plastic waste completely. On the contrary, residual ash can be considered a potential source of microplastic release into the atmosphere or environment [66]. If the plastics are completely incinerated, this process can produce airborne contaminants or pollutants [66]. As demonstrated, incomplete PVA breakdown within conventional water treatment results in a fraction of the material being sequestered within biosolids. The effects and behavior of residual PVA particles within biosolids are not well understood. More research is required to determine their impact on the environment relative to other, more conventional, plastics, whose physical presence in biosolids and ability to adsorb dangerous contaminants creates a threat to ecosystems.

In summary, this research aimed to isolate trends within the current industrial output of PVA used for laundry or dish detergent pods in US wastewater; investigate the components; assess the biodegradability, solubility, and bacterial effect on its structure; and, lastly, outline the potential risks PVA poses as an environmental pollutant. We observed that PVA has low degradation rates within WWTPs; thus, its hydrophilicity and massive production numbers make it a cause for concern as a pollutant in the natural environment. Very little research exists that aims to monitor the biodegradability of PVA in the natural environment. This presents a challenge in determining its role or impact as a pollutant. Research into truly eco-friendly substitutes for PVA is warranted and should be further explored. Improving upon this research is essential for better understanding the link between PVA usage, and public and environmental health.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/ijerph18116027/s1>, Literature review, survey questions, discussion of PVA degrading bacteria, Figure S1: Flow chart flow diagram, Table S1: Bacteria types and percent PVA degradation, Table S2: Soil PVA degradation rates, Table S3: Hydraulic retention times and solid retention times for WWTP segments, PVA Toxicity analysis.

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Article

Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk

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Abstract: The widespread use of plastics determines the inevitable human exposure to its by-products, including microplastics (MPs), which enter the human organism mainly by ingestion, inhalation, and dermal contact. Once internalised, MPs may pass across cell membranes and translocate to different body sites, triggering specific cellular mechanisms. Hence, the potential health impairment caused by the internalisation and accumulation of MPs is of prime concern, as confirmed by numerous studies reporting evident toxic effects in various animal models, marine organisms, and human cell lines. In this pilot single-centre observational prospective study, human breastmilk samples collected from N. 34 women were analysed by Raman Microspectroscopy, and, for the first time, MP contamination was found in 26 out of 34 samples. The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition. The most abundant MPs were composed of polyethylene, polyvinyl chloride, and polypropylene, with sizes ranging from 2 to 12 µm. MP data were statistically analysed in relation to specific patients' data (age, use of personal care products containing plastic compounds, and consumption of fish/shellfish, beverages, and food in plastic packaging), but no significant relationship was found, suggesting that the ubiquitous MP presence makes human exposure inevitable.

Keywords: microplastics; human breastmilk; Raman microspectroscopy; infants' nutrition

1. Introduction

The global production of plastics has reached the impressive amount of more than 350 million tons per year. This is the result of the massive demand for this material, which has been considered, until now, the golden choice in terms of durability, usability, and versatility for a huge variety of applications and consumer products [1–3]. This widespread use of plastics also led to their accumulation in landfills and in the natural environment. In fact, as a consequence of the extensive production and employment of single-use products, which represent >40% of manufactured plastics, 250,000 tons of plastic litter is estimated to be floating in the oceans [4]. Although several countries are introducing new regulations on plastic waste management and recycling strategies, it has to be noted that in 2018 in Europe, only 32.5% of post-consumer waste plastic was recycled, while 24.9% was accumulated in landfills [5].

Environmental plastic contamination derives from several factors, including mis-managed plastic waste, fishing nets in the sea, and different household and commercial activities, such as washing synthetic textiles, road markings, tires, marine coatings, personal care products, and plastic pellets [6,7]. In particular, after being released into the environment, plastic products undergo a degradation process caused by the action of atmospheric agents, such as waves, abrasion, UV radiation, and photo-oxidation, in combination with biological processes, which leads to the formation of microplastics (MPs) [8]. MPs range from 5 millimetres to 100 nanometres and are classified as primary or secondary based on their source of release into the environment: primary MPs are purposely manufactured at sizes <5 mm to be employed for commercial purposes (such as glitter in cosmetic products and microbeads in cleansers, scrubs, and dish scrubbing pads), while secondary MPs are generated by the previously described environmental degradation processes of larger plastic items [9,10].

The ubiquitous occurrence of MPs in the environment determines inevitable human exposure, mainly by three routes: ingestion, inhalation, and dermal contact. Among all of them, ingestion is considered the major route, with an estimated intake of $39 \div 52$ thousand MPs per person per year [11–13]. Once internalised, MPs may pass across cell membranes [14], followed by accumulation or elimination by the onset of specific cellular mechanisms. All of these processes are mainly related to MPs' size, which cannot exceed 10–15 μm .

The potential health impairment caused by the internalisation and accumulation of MPs is of prime concern. Although information is still lacking on this topic, several studies reported evident toxic effects in various animal models, marine organisms, and human cell lines [15–17], showing that MPs, once internalised, are not inert as previously supposed and likely trigger local or systemic responses.

Given the strong concern related to the effects of MPs on animal and human health, the use of reliable and objective techniques for MP detection and characterisation is crucial. Among all of the exploited techniques, Raman Microspectroscopy (RMS) can be considered the gold standard, since it lets researchers characterise not only the morphological features of microparticles but also their chemical composition in terms of both polymer matrices and pigments. Moreover, RMS presents the advantage of enabling the analysis of MPs as small as $\sim 2 \mu\text{m}$ directly on filtration membranes, thanks to the high potential of light scattering [18–20]. Recently, our research group, for the first time, detected the presence of MPs in human placenta samples; this study, carried out by Raman Microspectroscopy, received extensive attention, since the delicate role played by this organ may be perturbed by the presence of MPs [21].

Based on these impressive results, we decided to investigate the contamination of microplastics in breastmilk to assess another MP exposure route in the extremely vulnerable population of infants. For this purpose, in the present study, milk samples collected from 34 consenting patients were analysed by Raman Microspectroscopy, and, for the first time, in most of the analysed samples, the presence of MPs was detected. The relevance of this research lies in breastmilk being the gold standard for infants' nutrition. Moreover, it reflects both the mother's and infant's postnatal exposure, and hence, it represents an optimal matrix for contaminant biomonitoring [22]. In fact, milk consists of protein and fat globules in a carbohydrate-based suspension and represents a favourable environment for the lipophilic nature of MPs and other chemicals. In this regard, it is noteworthy that several studies reported the contamination of breastmilk by phthalates, heavy metals, and perfluorinated compounds [23–25].

2. Materials and Methods

2.1. Cohort Selection

This was a pilot observational descriptive study in a prospective and single-centre cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021), and it was carried out in full accordance with ethical principles, including The

Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. A dedicated cohort of N. 34 patients, all characterised by pregnancies without complications, was enrolled at Ospedale Fatebenefratelli Isola Tiberina (Rome, Italy). Exclusion criteria were: (a) medically prescribed special diets within 4 weeks prior to delivery; (b) diarrhoea or severe constipation within 2 weeks prior to delivery; (c) use of antibiotics within 2 weeks prior to delivery; (d) use of drugs affecting intestinal resorption, e.g., activated charcoal or cholestyramine, within 2 weeks prior to delivery; (e) diagnosis of a gastrointestinal pathology, e.g., ulcerative colitis or Crohn's disease, except for appendectomy; (f) cancer, HIV, or any other serious illness demanding medical treatment; (g) invasive or abrasive dental treatments within 2 weeks prior to delivery; (h) alcohol abuse (defined as Alcohol Use Disorder Identification test >10); and (i) current or recent (within the previous 4 weeks) participation in a clinical trial. Patients who decided to participate were asked to sign an informed consent form. Patients were also asked to fill in a questionnaire to record their food consumption, with a special focus on fish, shellfish, and foods employing packaging, and the use of personal care products from 7 days before the expected date of delivery to 7 days after.

2.2. Sample Collection

Breastmilk samples were collected 1 week after delivery at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital (Rome, Italy). Patients were guided on a manual expression procedure, recommended by the World Health Organization and described in a document released by the Italian Ministry of Health [26], which uses manual expression to obtain the maximum milk output and to avoid pain or damage to the breast tissue. No breast pumps were allowed to avoid contamination from its plastic components. Briefly, the manual expression procedure consists of cupping the breast with one hand, with the other forming a C-shape with the thumb and the forefinger, 3–4 cm from the base of the nipple; then, pressure is applied by pushing towards the ribcage, squeezing with the thumb and forefinger, and finally releasing the pressure. The sequence of pressure, squeeze, and release was repeated until obtaining an adequate amount of milk. Milk samples were placed into glass flasks, weighed, and then stored at $-20\text{ }^{\circ}\text{C}$ until processing. Each sample contained an average amount of 4.16 ± 1.73 g of breastmilk.

2.3. Sample Digestion and Filtration

In order to remove organic components from milk samples, a digestion protocol was set up and performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). A 10% KOH solution prepared using $1.6\text{ }\mu\text{m}$ filtered deionised water and KOH tablets (Sigma-Aldrich) was added to each flask in a ratio of sample to KOH of 1:10 (*w/v*). Flasks were sealed and incubated at $40\text{ }^{\circ}\text{C}$ for 48 h [27]. Digestates were then filtered through a $1.6\text{ }\mu\text{m}$ pore-size filter membrane (Whatman GF/A) by a vacuum pump connected to a filter funnel. Filter membranes were dried at room temperature and stored in glass Petri dishes until Raman Microspectroscopy (RMS) analysis.

2.4. Detection and Identification of MPs by Raman Microspectroscopy

RMS analysis was performed by using an XploRA Nano Raman Microspectrometer (Horiba Scientific) at the ARI Laboratory of Università Politecnica delle Marche (Ancona, Italy). All filter membranes, including those deriving from the procedural blanks, were inspected by visible light using a $\times 10$ objective (Olympus MPLAN10 \times /0.25). The detected MPs were morphologically characterised by a $\times 100$ objective (Olympus MPLAN100 \times /0.90) and then directly analysed on the filter by RMS (spectral range 200–1800 cm^{-1} , 532 nm or 785 nm laser diode, 600 lines per mm grating). Spectra were dispersed onto a 16-bit dynamic range Peltier-cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. To reduce noise and enhance spectrum quality, raw

Raman spectra were subjected to polynomial baseline correction and vector normalisation (LabSpec 6 software, Horiba Scientific). The polymer matrix of the detected particles was identified by comparing the collected Raman spectra with spectral libraries of polymers and pigments obtained by measuring standard polymers/compounds (KnowItAll software, John Wiley & Sons, Inc., Hoboken, NJ, USA) [28,29]. Similarities of more than 80 of the Hit Quality Index (HQI) were considered satisfactory.

2.5. Quality Assurance and Control (QA/QC)

Efforts were adopted to avoid microplastic contamination during sample collection, storage, processing, and analysis. To this aim, a plastic-free protocol was adopted during all phases of the experiment, and a dedicated room was used for the digestion of milk samples, filtration, and RMS analysis steps. Routinely employed plastic tools were replaced with sterilised glass ones. Cotton laboratory coats and single-use latex gloves were worn during all phases of the experiment. All liquids, including ethanol for cleaning and deionised water for cleaning and preparation of all solutions, were filtered through 1.6 µm pore-size filter membranes (Whatman GF/A). Work surfaces were thoroughly washed with 70% ethanol prior to starting all procedures and during the experimental time. Glassware and instruments, including scissors and tweezers, were washed using dishwashing liquid, triple rinsed with 70% ethanol, and finally rinsed with 1.6 µm filtered deionised water.

Moreover, environmental and procedural blanks were prepared and thoroughly analysed to detect microplastic contamination deriving from the laboratory environment and from other external sources. As regards environmental blanks, a filter membrane soaked with 1.6 µm filtered deionised water was placed into an uncovered Petri dish and positioned each day in the above-mentioned dedicated room. A procedural blank was also prepared together with every batch of samples following the exact same procedure as samples, but without adding milk. The filters deriving from environmental and procedural blanks were first inspected by stereomicroscope.

2.6. Statistical Analysis

Data analysis was performed by using the statistical software package Prism6 (Graphpad Software, Inc., San Diego, CA, USA). Normality was checked by the D'Agostino and Pearson omnibus normality test. Chi-square test, Student's *t*-test, and one-way analysis of variance (ANOVA) were performed to compare data accordingly. The significance threshold was set at $p < 0.05$.

3. Results

In the present study, N. 34 breastmilk samples were investigated by RMS for the presence of microplastics. MP contamination was found in 26 out of 34 women. As regards QA/QC protocols, the analysis of environmental (N. 14) and procedural (N. 9) blanks was performed. In the environmental blanks, only fibres, for a total of N. 16, ranging from 571 µm to 3000 µm, were found. Conversely, no MP contamination was found in the filters from the procedural blanks. Given the dimensions of the fibres, which are not compatible with the translocation into breastmilk, and the absence of fibres in the analysed milk samples, there was no need to blank-correct the results.

All details about the identified microparticles (such as morphology, dimensions, colour, polymer matrix, and pigment) are listed in Table 1.

Table 1. Information about patients (age, quantity of milk sample, and abundance of MPs) and morphological and chemical features of the identified microparticles.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#1	28	8.44	2	0.24	irregular fragment	~10 µm	orange	nitrocellulose	
					irregular fragment	~6 µm	orange	polyethylene	
#2	32	4.92	1	0.20	irregular fragment	~3 µm	blue	polyethylene	
#3	32	5.83	1	0.17	irregular fragment	~6 µm	black	polyvinyl chloride	
#4	38	5.09	2	0.39	irregular fragment	~2 µm	red	polyvinyl chloride	
					irregular fragment	~3 µm	blue	polypropylene	
#5	36	7.08	2	0.28	irregular fragment	~6 µm	red	chlorinated polyethylene	
					sphere	~5 µm	grey	polypropylene	
#6	40	1.95	4	2.05	irregular fragment	~5 µm	light blue	polyvinyl chloride	
					irregular fragment	~1 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
#7	38	3.49	5	1.43	sphere	~5 µm	brown	polyvinyl alcohol	
					irregular fragment	~3 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	brown/grey	nitrocellulose	
					sphere	~2 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue	polypropylene	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#8	36	4.21	2	0.47	irregular fragment	~2 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~10 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
#9	45	2.81	3	1.07	irregular fragment	~4 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~5 µm	yellow/orange	polyethylene	
					irregular fragment	~3 µm	light blue	polyvinyl chloride	
#10	34	5.58	2	0.36	irregular fragment	~6 µm	orange	polyethylene	
					irregular fragment	~6 µm	blue	polypropylene	
#11	39	2.32	0	0					
#12	32	3.55	2	0.56	irregular fragment	~2 µm	black	polyethylene	
					irregular fragment	~10 µm	green	poly(ethylene-co-vinyl acetate)	Pigment Green 7 (C.I. Constitution 74260)
#13	41	2.76	1	0.36	irregular fragment	~12 µm	blue	polyethylene	
#14	50	4.50	3	0.67	irregular fragment	~3 µm	brown		Pigment Yellow 43 / Brown 6 (C.I. Constitution 77492)
					irregular fragment	~3 µm	light blue	polyvinyl chloride	
					sphere	~4 µm	grey	polypropylene	
#15	33	5.99	2	0.33	irregular fragment	~5 µm	orange	polyethylene	
					irregular fragment	~6 µm	red	polyvinyl chloride	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#16	37	4.65	2	0.43	irregular fragment	~10 µm	blue	polyethylene	
					irregular fragment	~2 µm	transparent	polyethylene	
#17	32	1.64	2	1.22	sphere	~5 µm	transparent	polyethylene	
					irregular fragment	~5 µm	brown		Pigment Red 101/102 (C.I. Constitution 77491)
#18	41	3.10	1	0.32	irregular fragment	~8 µm	black	poly(ethyl methacrylate)	
#19	38	6.06	2	0.33	irregular fragment	~8 µm	orange	nitrocellulose	
					irregular fragment	~2 µm	blue/green	polypropylene	
#20	37	4.19	3	0.71	irregular fragment	~3 µm	magenta	polyvinyl chloride	
					irregular fragment	~12 µm	light blue	polyvinyl chloride	
					irregular fragment	~12 µm	green	polyvinyl chloride	
#21	40	2.36	1	0.42	irregular fragment	~5 µm	blue	acrylonitrile butadiene styrene	
#22	41	5.47	0	0					
#23	35	3.82	0	0					
#24	48	2.53	5	1.98	irregular fragment	~2 µm	orange	polystyrene	
					irregular fragment	~10 µm	yellow	polyethylene	
					irregular fragment	~12 µm	transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
					irregular fragment	~5 µm	brown	polyester	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#25	39	7.51	1	0.13	irregular fragment	~10 µm	blue	polyamide	
#26	37	5.65	2	0.35	irregular fragment	~6 µm	white/transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
#27	31	3.06	1	0.33	irregular fragment	~5 µm	brown	polycarbonate	
#28	35	3.54	0	0					
#29	35	2.68	1	0.37	irregular fragment	~2 µm	light blue	polyethylene	
#30	47	1.84	5	2.72	irregular fragment	~7 µm	white/transparent	polyethylene	
					irregular fragment	~8 µm	yellow/brown	polyethylene	
					irregular fragment	~2 µm	white	polyethylene	
					irregular fragment	~4 µm	orange	high-density polyethylene	
					irregular fragment	~4 µm	blue	polyvinyl chloride	
#31	49	3.85	0	0					
#32	42	4.21	0	0					
#33	45	5.10	0	0					
#34	42	1.54	0	0					

For clarity, in Figure 1, the microphotographs and corresponding Raman spectra of some selected MPs found in the analysed samples are reported.

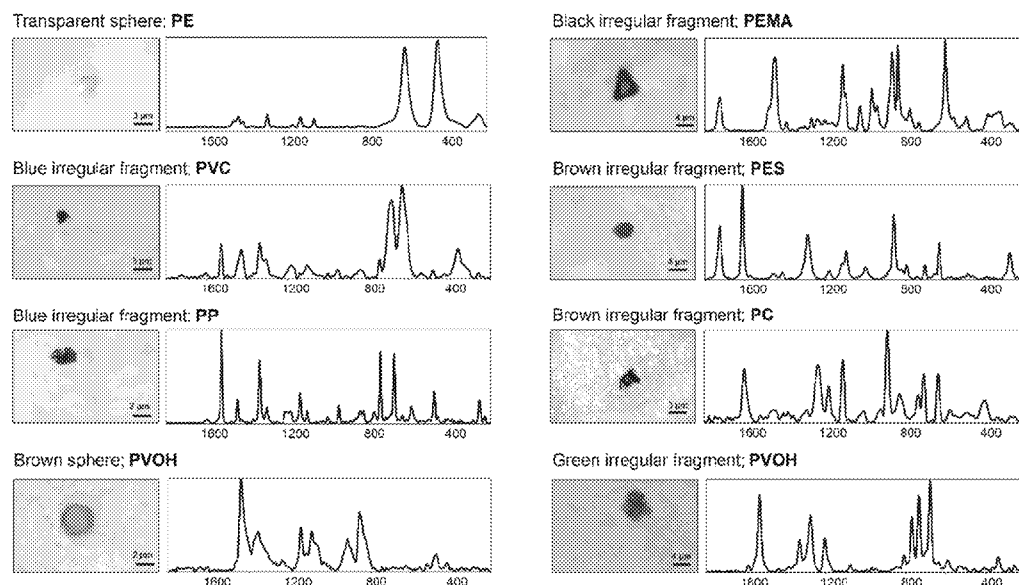


Figure 1. Microphotographs and Raman spectra (wavenumbers, cm^{-1}) of some selected MPs found in the analysed breastmilk samples. PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); PES: polyester, and PC: polycarbonate.

The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition (Figure 2). As regards the shape, only irregular fragments and spheres were found, while no films or fibres were identified (Figure 2A). Moreover, most of the identified MPs were pigmented (ca. 90%), with blue and orange/yellow being the most abundant colours (ca. 36% and ca. 17%, respectively; Figure 2B). As regards MPs' dimensions, almost half of them (ca. 47%) were in the range of 4–9 μm ; ca. 29% were $\leq 3 \mu\text{m}$, while ca. 24% were $\geq 10 \mu\text{m}$ (Figure 2C). For 48 out of the total 58 identified MPs, the polymer matrix was also identified, while for the remaining ones, the contribution of the pigments used for plastic staining was the only signal in the collected Raman spectra [21,30,31]. Within the identified polymer matrices, the most abundant ones were polyethylene (PE, 38%), polyvinyl chloride (PVC, 21%), and polypropylene (PP, 17%) (Figure 2D).

The statistical relationship between data related to patients and the presence/number of MPs was also investigated. In particular, the following patient-related parameters were analysed: patient's age; use of personal care products containing plastic compounds (including lotions, soaps, and toothpaste); and consumption, in the 7 days prior to the expected date of delivery and 7 days after, of fish/shellfish, beverages in plastic bottles, and food in plastic packaging. In Figure 3A–E, the statistical analysis of the percentages of women with and without MPs in their milk, divided according to each of the above-defined parameters, is reported. As regards 'patient's age', women were divided into three groups as follows: ≤ 35 years old, 36–40 years old, and ≥ 41 years old; no statistically significant difference was observed among groups ($p = 0.648$; Figure 3A). Moreover, women's habits of food consumption and use of personal care products were investigated, and in this case as well, no statistically significant difference among groups was observed ('fish/shellfish consumption', $p = 0.961$, Figure 3B; 'personal care product with plastics', $p = 0.1611$, Figure 3C; 'beverages in plastic bottles', $p = 0.9107$, Figure 3D; and 'food in plastic packaging', $p = 0.2963$, Figure 3E). For a deeper analysis, each of the above-defined parameters was also considered in relation to the number of detected MPs (Figure 3F–J); as expected, no significant difference among groups was revealed (one-way ANOVA or Student's *t*-test).

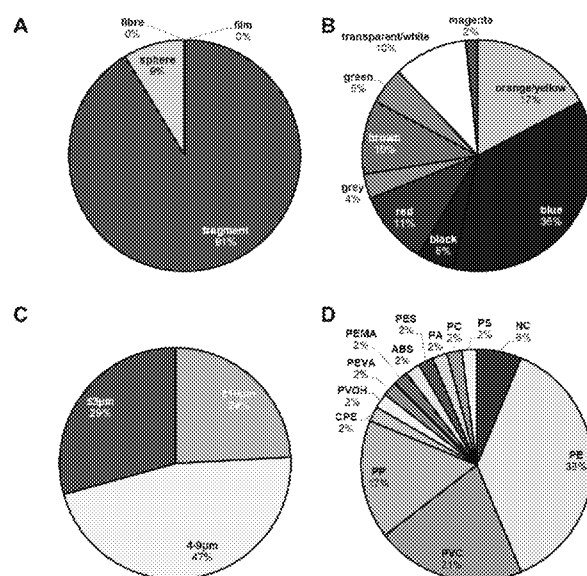


Figure 2. Percentage abundances of identified shapes (A), colours (B), dimensions (C), and polymer matrices (D). PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; CPE: chlorinated polyethylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); ABS: acrylonitrile butadiene styrene; PES: polyester; PA: polyamide; PC: polycarbonate; PS: polystyrene, and NC: nitrocellulose.

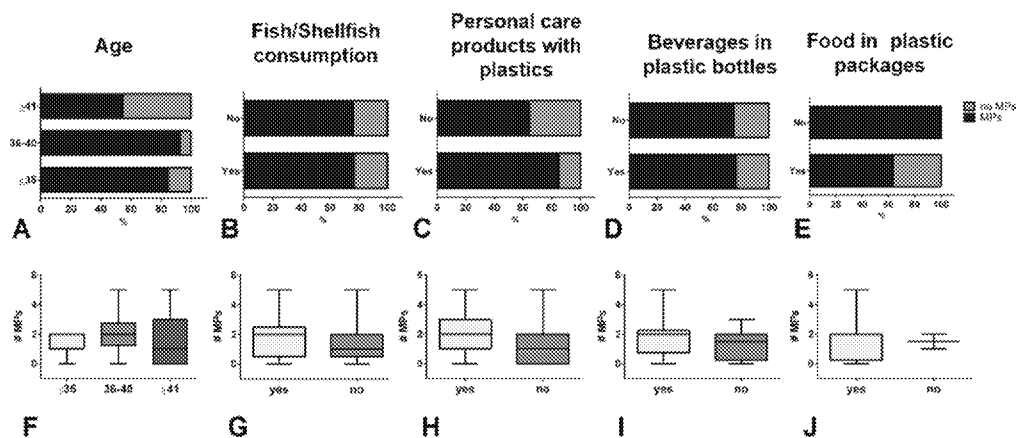


Figure 3. Percentage abundances of samples with (MPs) and without (no MPs) microplastics, divided according to the following selected parameters: (A) age of patient; (B) consumption of fish/shellfish in the 7 days prior to the expected date of delivery and 7 days after; (C) use of personal care products with plastic compounds in the 7 days prior to the expected date of delivery and 7 days after; (D) consumption of beverages in plastic bottles in the 7 days prior to the expected date of delivery and 7 days after, and (E) consumption of food in plastic packaging in the 7 days prior to the expected date of delivery and 7 days after. Number of identified microplastics divided according to the above-defined parameters (F–J) (box charts: centre line marks the median, edges indicate the 5th and 95th percentiles, and whiskers indicate the minimum and maximum values).

4. Discussion

Breastmilk represents the best standard nutrition for infants, thanks to its provision of nutrients and enhancement of the immune system [24]. Hence, assessing its quality in terms of possible contamination is mandatory. In fact, mothers are exposed daily to a great variety of chemicals present in the environment, for example, through food, beverages, and personal care products, and for this reason, breastmilk may be contaminated by these compounds, likely impacting children's health [32]. To date, the presence of

polychlorinated bisphenyls (PCBs), organochlorine pesticides, polybrominated diphenyl esters (PBDEs), phthalates and phthalate metabolites, per- and polyfluoroalkyl substances (PFASs), phenols, and metals have been detected in human milk [33]. In fact, since most of these contaminants are lipophilic and have a tendency to deposit in adipose tissue, they may be translocated to milk during lactation [34,35]. It is known that early life stages are the most sensitive to PCBs' toxic effects, which mainly consist of a severe impact on endocrine and cognitive systems, leading to reduced IQ and altered behaviour [36]. Similarly, PBDEs are recognised as neurotoxic, especially in children, with effects on motor, cognitive, and behavioural development [37]. Phthalates have been reported in the literature to negatively impact male reproductive functionality [38]; moreover, childhood exposure to phthalates was shown to increase the risk of allergic diseases and altered physical/neurocognitive development [39,40].

It is noteworthy that most of these environmental pollutants are also able to interact with MPs by several sorption mechanisms, which depend on polymer size, shape, density, colour, and chemical composition [41]; for example, phthalate esters have shown major sorption on polystyrene, polyethylene, and polyvinyl chloride microparticles [42]. Hence, since MPs are ubiquitous environmental contaminants and represent potential vectors for toxic organic compounds with known health impairment effects, their detection in biological matrices is actually of great concern [15–17].

In this study, for the first time, MPs were found in breastmilk samples; it should be stated that the number of microparticles that we detected could be underestimated, since only an aliquot of ~4 g of milk was considered for each sample. MPs were characterised by RMS and classified in terms of shape, dimensions, colour, and chemical composition. In this regard, almost all MPs were blue and orange/yellow irregular fragments with dimensions ranging from 2 μm to 12 μm , consistent with translocation mechanisms. In accordance with other studies reported in the literature, the most abundant polymers were polyethylene, polyvinyl chloride, and polypropylene [43,44].

Several MP routes of exposure have been reported in the literature, including inhalation, dermal contact, and ingestion, with the latter being considered the most impactful, with an estimated total intake of around 39–52 thousand per person per year [12,13]. Once ingested/inhaled, MPs can be internalised in human tissues [11]. At the gastrointestinal level, they may pass through the epithelium by endocytosis mechanisms or by paracellular diffusion, after which they are translocated by dendritic cells through the lymphatic circulation and reach the circulatory system [11]. As regards the respiratory system, inhaled MPs likely penetrate the lower respiratory tract, characterised by a thin mucus layer, and spread into the bloodstream after cellular uptake or paracellular diffusion [45].

Currently, there is growing scientific evidence about MPs in humans. Schwabl et al. reported the detection of MPs in human stool [46], while, as a further measure, Ibrahim et al. described the presence of MPs in human colectomy samples, proving that MPs in part cross the intestinal barrier [47]. As evidence of inhalation exposure, Amato-Lourenço et al. detected, in human lung tissue, <5.5 μm polymeric MPs and fibres ranging from 8.12 to 16.8 μm [48]. We recently found MPs in the human placenta, which represents the interface between the foetus and the mother exposed to the external environment [21], results that were also confirmed by Braun et al. [49]. Very recently, the presence of plastic particles in human blood finally proved the transport of MPs in the bloodstream to every body site [50].

As regards the mammary gland, two hypothetical pathways have been suggested for the translocation of exogenous particles from the bloodstream to breast milk: the mammary epithelial cell-dependent and the immune cell-dependent pathways, with the latter being particularly relevant in the case of inhaled particles [23–25,51,52]. Hence, a possible association between the presence of MPs in breastmilk and specific information regarding mothers' habits (such as the consumption of fish and shellfish, beverages in plastic bottles, and food in plastic packaging and the use of personal care products containing plastic compounds in the 7 days prior to the expected date of delivery and the 7 days after) was

investigated, but no relationship was found between MP presence/number and each of the above-mentioned parameters.

The lack of association with the use of personal care products is likely explained by considering that dermal contact has a minor impact as an exposure route, since only particles <100 nanometres can cross the dermal barrier [11]. Conversely, the absence of a relation with mothers' food habits is more difficult to explain, since the major route of MP exposure is represented by ingestion. In fact, numerous food-related sources of MPs have been reported, including fish, shellfish, and human essential daily consumables, such as table salt, sugar, bottled water, milk, honey, plastic teabags, and to a greater extent, plastic kitchen tools, plates, and packaging [53]. Hence, our findings suggest that, since MPs are ubiquitous in the environment, exposure to these microparticles is inevitable, and, for this reason, it is impossible to isolate a specific source among the complex set of faced exposures.

5. Conclusions

The evidence of MPs in human breastmilk, coupled with the previous discovery of these microparticles in the human placenta, represents a great concern, since it impacts the extremely vulnerable population of infants. In fact, the chemicals possibly contained in foods, beverages, and personal care products consumed by breastfeeding mothers may be transferred to the offspring, potentially exerting a toxic effect. Hence, it is mandatory to increase efforts in scientific research to deepen the knowledge of the potential health impairment caused by MP internalisation and accumulation, especially in infants, and to assess innovative, useful ways to reduce exposure to these contaminants during pregnancy and lactation.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk

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Abstract: The widespread use of plastics determines the inevitable human exposure to its by-products, including microplastics (MPs), which enter the human organism mainly by ingestion, inhalation, and dermal contact. Once internalised, MPs may pass across cell membranes and translocate to different body sites, triggering specific cellular mechanisms. Hence, the potential health impairment caused by the internalisation and accumulation of MPs is of prime concern, as confirmed by numerous studies reporting evident toxic effects in various animal models, marine organisms, and human cell lines. In this pilot single-centre observational prospective study, human breastmilk samples collected from N. 34 women were analysed by Raman Microspectroscopy, and, for the first time, MP contamination was found in 26 out of 34 samples. The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition. The most abundant MPs were composed of polyethylene, polyvinyl chloride, and polypropylene, with sizes ranging from 2 to 12 µm. MP data were statistically analysed in relation to specific patients' data (age, use of personal care products containing plastic compounds, and consumption of fish/shellfish, beverages, and food in plastic packaging), but no significant relationship was found, suggesting that the ubiquitous MP presence makes human exposure inevitable.

Keywords: microplastics; human breastmilk; Raman microspectroscopy; infants' nutrition

1. Introduction

The global production of plastics has reached the impressive amount of more than 350 million tons per year. This is the result of the massive demand for this material, which has been considered, until now, the golden choice in terms of durability, usability, and versatility for a huge variety of applications and consumer products [1–3]. This widespread use of plastics also led to their accumulation in landfills and in the natural environment. In fact, as a consequence of the extensive production and employment of single-use products, which represent >40% of manufactured plastics, 250,000 tons of plastic litter is estimated to be floating in the oceans [4]. Although several countries are introducing new regulations on plastic waste management and recycling strategies, it has to be noted that in 2018 in Europe, only 32.5% of post-consumer waste plastic was recycled, while 24.9% was accumulated in landfills [5].

Environmental plastic contamination derives from several factors, including mis-managed plastic waste, fishing nets in the sea, and different household and commercial activities, such as washing synthetic textiles, road markings, tires, marine coatings, personal care products, and plastic pellets [6,7]. In particular, after being released into the environment, plastic products undergo a degradation process caused by the action of atmospheric agents, such as waves, abrasion, UV radiation, and photo-oxidation, in combination with biological processes, which leads to the formation of microplastics (MPs) [8]. MPs range from 5 millimetres to 100 nanometres and are classified as primary or secondary based on their source of release into the environment: primary MPs are purposely manufactured at sizes <5 mm to be employed for commercial purposes (such as glitter in cosmetic products and microbeads in cleansers, scrubs, and dish scrubbing pads), while secondary MPs are generated by the previously described environmental degradation processes of larger plastic items [9,10].

The ubiquitous occurrence of MPs in the environment determines inevitable human exposure, mainly by three routes: ingestion, inhalation, and dermal contact. Among all of them, ingestion is considered the major route, with an estimated intake of $39 \div 52$ thousand MPs per person per year [11–13]. Once internalised, MPs may pass across cell membranes [14], followed by accumulation or elimination by the onset of specific cellular mechanisms. All of these processes are mainly related to MPs' size, which cannot exceed 10–15 μm .

The potential health impairment caused by the internalisation and accumulation of MPs is of prime concern. Although information is still lacking on this topic, several studies reported evident toxic effects in various animal models, marine organisms, and human cell lines [15–17], showing that MPs, once internalised, are not inert as previously supposed and likely trigger local or systemic responses.

Given the strong concern related to the effects of MPs on animal and human health, the use of reliable and objective techniques for MP detection and characterisation is crucial. Among all of the exploited techniques, Raman Microspectroscopy (RMS) can be considered the gold standard, since it lets researchers characterise not only the morphological features of microparticles but also their chemical composition in terms of both polymer matrices and pigments. Moreover, RMS presents the advantage of enabling the analysis of MPs as small as $\sim 2 \mu\text{m}$ directly on filtration membranes, thanks to the high potential of light scattering [18–20]. Recently, our research group, for the first time, detected the presence of MPs in human placenta samples; this study, carried out by Raman Microspectroscopy, received extensive attention, since the delicate role played by this organ may be perturbed by the presence of MPs [21].

Based on these impressive results, we decided to investigate the contamination of microplastics in breastmilk to assess another MP exposure route in the extremely vulnerable population of infants. For this purpose, in the present study, milk samples collected from 34 consenting patients were analysed by Raman Microspectroscopy, and, for the first time, in most of the analysed samples, the presence of MPs was detected. The relevance of this research lies in breastmilk being the gold standard for infants' nutrition. Moreover, it reflects both the mother's and infant's postnatal exposure, and hence, it represents an optimal matrix for contaminant biomonitoring [22]. In fact, milk consists of protein and fat globules in a carbohydrate-based suspension and represents a favourable environment for the lipophilic nature of MPs and other chemicals. In this regard, it is noteworthy that several studies reported the contamination of breastmilk by phthalates, heavy metals, and perfluorinated compounds [23–25].

2. Materials and Methods

2.1. Cohort Selection

This was a pilot observational descriptive study in a prospective and single-centre cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 708/CE Lazio 1; 24 May 2021), and it was carried out in full accordance with ethical principles, including The

Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. A dedicated cohort of N. 34 patients, all characterised by pregnancies without complications, was enrolled at Ospedale Fatebenefratelli Isola Tiberina (Rome, Italy). Exclusion criteria were: (a) medically prescribed special diets within 4 weeks prior to delivery; (b) diarrhoea or severe constipation within 2 weeks prior to delivery; (c) use of antibiotics within 2 weeks prior to delivery; (d) use of drugs affecting intestinal resorption, e.g., activated charcoal or cholestyramine, within 2 weeks prior to delivery; (e) diagnosis of a gastrointestinal pathology, e.g., ulcerative colitis or Crohn's disease, except for appendectomy; (f) cancer, HIV, or any other serious illness demanding medical treatment; (g) invasive or abrasive dental treatments within 2 weeks prior to delivery; (h) alcohol abuse (defined as Alcohol Use Disorder Identification test >10); and (i) current or recent (within the previous 4 weeks) participation in a clinical trial. Patients who decided to participate were asked to sign an informed consent form. Patients were also asked to fill in a questionnaire to record their food consumption, with a special focus on fish, shellfish, and foods employing packaging, and the use of personal care products from 7 days before the expected date of delivery to 7 days after.

2.2. Sample Collection

Breastmilk samples were collected 1 week after delivery at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital (Rome, Italy). Patients were guided on a manual expression procedure, recommended by the World Health Organization and described in a document released by the Italian Ministry of Health [26], which uses manual expression to obtain the maximum milk output and to avoid pain or damage to the breast tissue. No breast pumps were allowed to avoid contamination from its plastic components. Briefly, the manual expression procedure consists of cupping the breast with one hand, with the other forming a C-shape with the thumb and the forefinger, 3–4 cm from the base of the nipple; then, pressure is applied by pushing towards the ribcage, squeezing with the thumb and forefinger, and finally releasing the pressure. The sequence of pressure, squeeze, and release was repeated until obtaining an adequate amount of milk. Milk samples were placed into glass flasks, weighed, and then stored at $-20\text{ }^{\circ}\text{C}$ until processing. Each sample contained an average amount of $4.16 \pm 1.73\text{ g}$ of breastmilk.

2.3. Sample Digestion and Filtration

In order to remove organic components from milk samples, a digestion protocol was set up and performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). A 10% KOH solution prepared using $1.6\text{ }\mu\text{m}$ filtered deionised water and KOH tablets (Sigma-Aldrich) was added to each flask in a ratio of sample to KOH of 1:10 (w/v). Flasks were sealed and incubated at $40\text{ }^{\circ}\text{C}$ for 48 h [27]. Digestates were then filtered through a $1.6\text{ }\mu\text{m}$ pore-size filter membrane (Whatman GF/A) by a vacuum pump connected to a filter funnel. Filter membranes were dried at room temperature and stored in glass Petri dishes until Raman Microspectroscopy (RMS) analysis.

2.4. Detection and Identification of MPs by Raman Microspectroscopy

RMS analysis was performed by using an XploRA Nano Raman Microspectrometer (Horiba Scientific) at the ARI Laboratory of Università Politecnica delle Marche (Ancona, Italy). All filter membranes, including those deriving from the procedural blanks, were inspected by visible light using a $\times 10$ objective (Olympus MPLAN10 \times /0.25). The detected MPs were morphologically characterised by a $\times 100$ objective (Olympus MPLAN100 \times /0.90) and then directly analysed on the filter by RMS (spectral range $200\text{--}1800\text{ cm}^{-1}$, 532 nm or 785 nm laser diode, 600 lines per mm grating). Spectra were dispersed onto a 16-bit dynamic range Peltier-cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. To reduce noise and enhance spectrum quality, raw

Raman spectra were subjected to polynomial baseline correction and vector normalisation (LabSpec 6 software, Horiba Scientific). The polymer matrix of the detected particles was identified by comparing the collected Raman spectra with spectral libraries of polymers and pigments obtained by measuring standard polymers/compounds (KnowItAll software, John Wiley & Sons, Inc., Hoboken, NJ, USA) [28,29]. Similarities of more than 80 of the Hit Quality Index (HQI) were considered satisfactory.

2.5. Quality Assurance and Control (QA/QC)

Efforts were adopted to avoid microplastic contamination during sample collection, storage, processing, and analysis. To this aim, a plastic-free protocol was adopted during all phases of the experiment, and a dedicated room was used for the digestion of milk samples, filtration, and RMS analysis steps. Routinely employed plastic tools were replaced with sterilised glass ones. Cotton laboratory coats and single-use latex gloves were worn during all phases of the experiment. All liquids, including ethanol for cleaning and deionised water for cleaning and preparation of all solutions, were filtered through 1.6 µm pore-size filter membranes (Whatman GF/A). Work surfaces were thoroughly washed with 70% ethanol prior to starting all procedures and during the experimental time. Glassware and instruments, including scissors and tweezers, were washed using dishwashing liquid, triple rinsed with 70% ethanol, and finally rinsed with 1.6 µm filtered deionised water.

Moreover, environmental and procedural blanks were prepared and thoroughly analysed to detect microplastic contamination deriving from the laboratory environment and from other external sources. As regards environmental blanks, a filter membrane soaked with 1.6 µm filtered deionised water was placed into an uncovered Petri dish and positioned each day in the above-mentioned dedicated room. A procedural blank was also prepared together with every batch of samples following the exact same procedure as samples, but without adding milk. The filters deriving from environmental and procedural blanks were first inspected by stereomicroscope.

2.6. Statistical Analysis

Data analysis was performed by using the statistical software package Prism6 (Graphpad Software, Inc., San Diego, CA, USA). Normality was checked by the D'Agostino and Pearson omnibus normality test. Chi-square test, Student's *t*-test, and one-way analysis of variance (ANOVA) were performed to compare data accordingly. The significance threshold was set at $p < 0.05$.

3. Results

In the present study, N. 34 breastmilk samples were investigated by RMS for the presence of microplastics. MP contamination was found in 26 out of 34 women. As regards QA/QC protocols, the analysis of environmental (N. 14) and procedural (N. 9) blanks was performed. In the environmental blanks, only fibres, for a total of N. 16, ranging from 571 µm to 3000 µm, were found. Conversely, no MP contamination was found in the filters from the procedural blanks. Given the dimensions of the fibres, which are not compatible with the translocation into breastmilk, and the absence of fibres in the analysed milk samples, there was no need to blank-correct the results.

All details about the identified microparticles (such as morphology, dimensions, colour, polymer matrix, and pigment) are listed in Table 1.

Table 1. Information about patients (age, quantity of milk sample, and abundance of MPs) and morphological and chemical features of the identified microparticles.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#1	28	8.44	2	0.24	irregular fragment	~10 µm	orange	nitrocellulose	
					irregular fragment	~6 µm	orange	polyethylene	
#2	32	4.92	1	0.20	irregular fragment	~3 µm	blue	polyethylene	
#3	32	5.83	1	0.17	irregular fragment	~6 µm	black	polyvinyl chloride	
#4	38	5.09	2	0.39	irregular fragment	~2 µm	red	polyvinyl chloride	
					irregular fragment	~3 µm	blue	polypropylene	
#5	36	7.08	2	0.28	irregular fragment	~6 µm	red	chlorinated polyethylene	
					sphere	~5 µm	grey	polypropylene	
#6	40	1.95	4	2.05	irregular fragment	~5 µm	light blue	polyvinyl chloride	
					irregular fragment	~1 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
#7	38	3.49	5	1.43	sphere	~5 µm	brown	polyvinyl alcohol	
					irregular fragment	~3 µm	light blue		Pigment Green 7 (C.I. Constitution 74260)
					irregular fragment	~10 µm	brown/grey	nitrocellulose	
					sphere	~2 µm	blue		Pigment Blue 29 (C.I. Constitution 77007)
					irregular fragment	~10 µm	light blue	polypropylene	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#8	36	4.21	2	0.47	irregular fragment	~2 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~10 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
#9	45	2.81	3	1.07	irregular fragment	~4 µm	red		Pigment Red 101/102 (C.I. Constitution 77491)
					irregular fragment	~5 µm	yellow/orange	polyethylene	
					irregular fragment	~3 µm	light blue	polyvinyl chloride	
#10	34	5.58	2	0.36	irregular fragment	~6 µm	orange	polyethylene	
					irregular fragment	~6 µm	blue	polypropylene	
#11	39	2.32	0	0					
#12	32	3.55	2	0.56	irregular fragment	~2 µm	black	polyethylene	
					irregular fragment	~10 µm	green	poly(ethylene-co-vinyl acetate)	Pigment Green 7 (C.I. Constitution 74260)
#13	41	2.76	1	0.36	irregular fragment	~12 µm	blue	polyethylene	
#14	50	4.50	3	0.67	irregular fragment	~3 µm	brown		Pigment Yellow 43 / Brown 6 (C.I. Constitution 77492)
					irregular fragment	~3 µm	light blue	polyvinyl chloride	
					sphere	~4 µm	grey	polypropylene	
#15	33	5.99	2	0.33	irregular fragment	~5 µm	orange	polyethylene	
					irregular fragment	~6 µm	red	polyvinyl chloride	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#16	37	4.65	2	0.43	irregular fragment	~10 µm	blue	polyethylene	
					irregular fragment	~2 µm	transparent	polyethylene	
#17	32	1.64	2	1.22	sphere	~5 µm	transparent	polyethylene	
					irregular fragment	~5 µm	brown		Pigment Red 101/102 (C.I. Constitution 77491)
#18	41	3.10	1	0.32	irregular fragment	~8 µm	black	poly(ethyl methacrylate)	
#19	38	6.06	2	0.33	irregular fragment	~8 µm	orange	nitrocellulose	
					irregular fragment	~2 µm	blue/green	polypropylene	
#20	37	4.19	3	0.71	irregular fragment	~3 µm	magenta	polyvinyl chloride	
					irregular fragment	~12 µm	light blue	polyvinyl chloride	
					irregular fragment	~12 µm	green	polyvinyl chloride	
#21	40	2.36	1	0.42	irregular fragment	~5 µm	blue	acrylonitrile butadiene styrene	
#22	41	5.47	0	0					
#23	35	3.82	0	0					
#24	48	2.53	5	1.98	irregular fragment	~2 µm	orange	polystyrene	
					irregular fragment	~10 µm	yellow	polyethylene	
					irregular fragment	~12 µm	transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
					irregular fragment	~5 µm	brown	polyester	

Table 1. Cont.

Patient	Age	Milk Quantity (g)	N. of MPs	MP/g	Shape	Size	Colour	Polymer Matrix	Pigment
#25	39	7.51	1	0.13	irregular fragment	~10 µm	blue	polyamide	
#26	37	5.65	2	0.35	irregular fragment	~6 µm	white/transparent	polyethylene	
					irregular fragment	~4 µm	light blue	polypropylene	
#27	31	3.06	1	0.33	irregular fragment	~5 µm	brown	polycarbonate	
#28	35	3.54	0	0					
#29	35	2.68	1	0.37	irregular fragment	~2 µm	light blue	polyethylene	
#30	47	1.84	5	2.72	irregular fragment	~7 µm	white/transparent	polyethylene	
					irregular fragment	~8 µm	yellow/brown	polyethylene	
					irregular fragment	~2 µm	white	polyethylene	
					irregular fragment	~4 µm	orange	high-density polyethylene	
					irregular fragment	~4 µm	blue	polyvinyl chloride	
#31	49	3.85	0	0					
#32	42	4.21	0	0					
#33	45	5.10	0	0					
#34	42	1.54	0	0					

For clarity, in Figure 1, the microphotographs and corresponding Raman spectra of some selected MPs found in the analysed samples are reported.

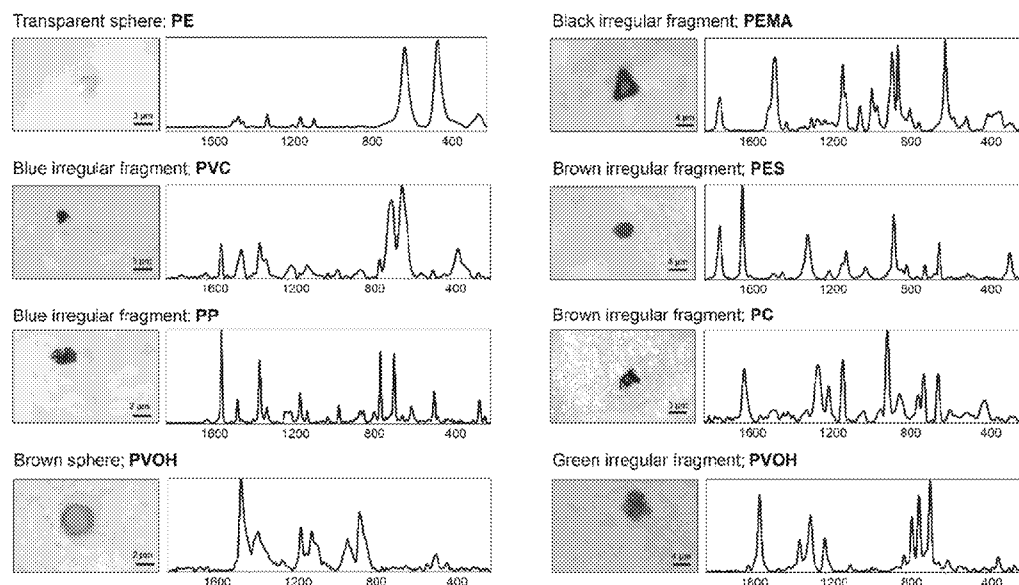


Figure 1. Microphotographs and Raman spectra (wavenumbers, cm^{-1}) of some selected MPs found in the analysed breastmilk samples. PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); PES: polyester, and PC: polycarbonate.

The detected microparticles were classified according to their shape, colour, dimensions, and chemical composition (Figure 2). As regards the shape, only irregular fragments and spheres were found, while no films or fibres were identified (Figure 2A). Moreover, most of the identified MPs were pigmented (ca. 90%), with blue and orange/yellow being the most abundant colours (ca. 36% and ca. 17%, respectively; Figure 2B). As regards MPs' dimensions, almost half of them (ca. 47%) were in the range of 4–9 μm ; ca. 29% were $\leq 3 \mu\text{m}$, while ca. 24% were $\geq 10 \mu\text{m}$ (Figure 2C). For 48 out of the total 58 identified MPs, the polymer matrix was also identified, while for the remaining ones, the contribution of the pigments used for plastic staining was the only signal in the collected Raman spectra [21,30,31]. Within the identified polymer matrices, the most abundant ones were polyethylene (PE, 38%), polyvinyl chloride (PVC, 21%), and polypropylene (PP, 17%) (Figure 2D).

The statistical relationship between data related to patients and the presence/number of MPs was also investigated. In particular, the following patient-related parameters were analysed: patient's age; use of personal care products containing plastic compounds (including lotions, soaps, and toothpaste); and consumption, in the 7 days prior to the expected date of delivery and 7 days after, of fish/shellfish, beverages in plastic bottles, and food in plastic packaging. In Figure 3A–E, the statistical analysis of the percentages of women with and without MPs in their milk, divided according to each of the above-defined parameters, is reported. As regards 'patient's age', women were divided into three groups as follows: ≤ 35 years old, 36–40 years old, and ≥ 41 years old; no statistically significant difference was observed among groups ($p = 0.648$; Figure 3A). Moreover, women's habits of food consumption and use of personal care products were investigated, and in this case as well, no statistically significant difference among groups was observed ('fish/shellfish consumption', $p = 0.961$, Figure 3B; 'personal care product with plastics', $p = 0.1611$, Figure 3C; 'beverages in plastic bottles', $p = 0.9107$, Figure 3D; and 'food in plastic packaging', $p = 0.2963$, Figure 3E). For a deeper analysis, each of the above-defined parameters was also considered in relation to the number of detected MPs (Figure 3F–J); as expected, no significant difference among groups was revealed (one-way ANOVA or Student's *t*-test).

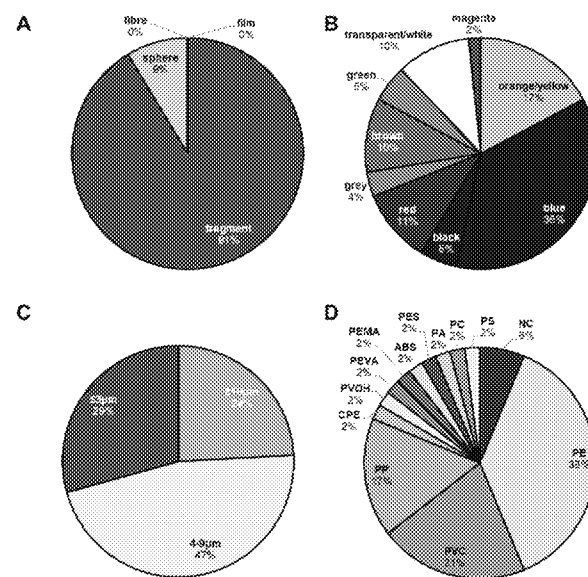


Figure 2. Percentage abundances of identified shapes (A), colours (B), dimensions (C), and polymer matrices (D). PE: polyethylene; PVC: polyvinyl chloride; PP: polypropylene; CPE: chlorinated polyethylene; PVOH: polyvinyl alcohol; PEVA: poly(ethylene-co-vinyl acetate); PEMA: poly(ethyl methacrylate); ABS: acrylonitrile butadiene styrene; PES: polyester; PA: polyamide; PC: polycarbonate; PS: polystyrene, and NC: nitrocellulose.

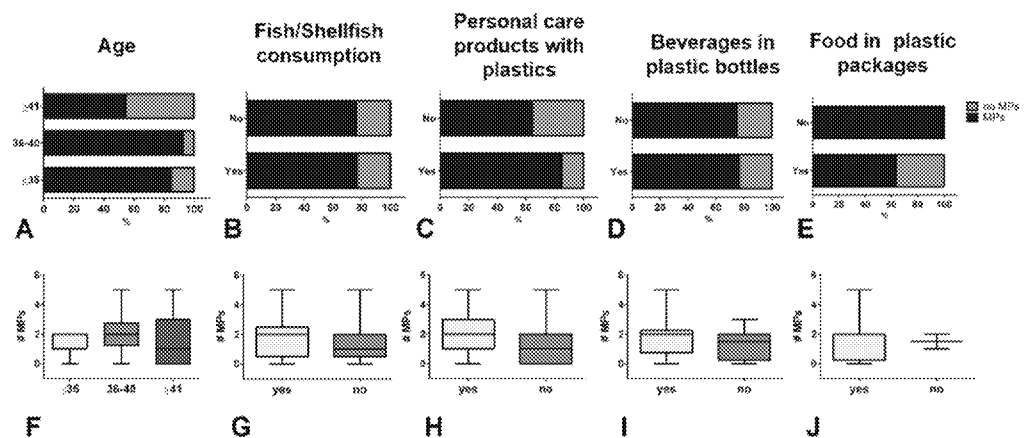


Figure 3. Percentage abundances of samples with (MPs) and without (no MPs) microplastics, divided according to the following selected parameters: (A) age of patient; (B) consumption of fish/shellfish in the 7 days prior to the expected date of delivery and 7 days after; (C) use of personal care products with plastic compounds in the 7 days prior to the expected date of delivery and 7 days after; (D) consumption of beverages in plastic bottles in the 7 days prior to the expected date of delivery and 7 days after, and (E) consumption of food in plastic packaging in the 7 days prior to the expected date of delivery and 7 days after. Number of identified microplastics divided according to the above-defined parameters (F–J) (box charts: centre line marks the median, edges indicate the 5th and 95th percentiles, and whiskers indicate the minimum and maximum values).

4. Discussion

Breastmilk represents the best standard nutrition for infants, thanks to its provision of nutrients and enhancement of the immune system [24]. Hence, assessing its quality in terms of possible contamination is mandatory. In fact, mothers are exposed daily to a great variety of chemicals present in the environment, for example, through food, beverages, and personal care products, and for this reason, breastmilk may be contaminated by these compounds, likely impacting children's health [32]. To date, the presence of

polychlorinated bisphenyls (PCBs), organochlorine pesticides, polybrominated diphenyl esters (PBDEs), phthalates and phthalate metabolites, per- and polyfluoroalkyl substances (PFASs), phenols, and metals have been detected in human milk [33]. In fact, since most of these contaminants are lipophilic and have a tendency to deposit in adipose tissue, they may be translocated to milk during lactation [34,35]. It is known that early life stages are the most sensitive to PCBs' toxic effects, which mainly consist of a severe impact on endocrine and cognitive systems, leading to reduced IQ and altered behaviour [36]. Similarly, PBDEs are recognised as neurotoxic, especially in children, with effects on motor, cognitive, and behavioural development [37]. Phthalates have been reported in the literature to negatively impact male reproductive functionality [38]; moreover, childhood exposure to phthalates was shown to increase the risk of allergic diseases and altered physical/neurocognitive development [39,40].

It is noteworthy that most of these environmental pollutants are also able to interact with MPs by several sorption mechanisms, which depend on polymer size, shape, density, colour, and chemical composition [41]; for example, phthalate esters have shown major sorption on polystyrene, polyethylene, and polyvinyl chloride microparticles [42]. Hence, since MPs are ubiquitous environmental contaminants and represent potential vectors for toxic organic compounds with known health impairment effects, their detection in biological matrices is actually of great concern [15–17].

In this study, for the first time, MPs were found in breastmilk samples; it should be stated that the number of microparticles that we detected could be underestimated, since only an aliquot of ~4 g of milk was considered for each sample. MPs were characterised by RMS and classified in terms of shape, dimensions, colour, and chemical composition. In this regard, almost all MPs were blue and orange/yellow irregular fragments with dimensions ranging from 2 µm to 12 µm, consistent with translocation mechanisms. In accordance with other studies reported in the literature, the most abundant polymers were polyethylene, polyvinyl chloride, and polypropylene [43,44].

Several MP routes of exposure have been reported in the literature, including inhalation, dermal contact, and ingestion, with the latter being considered the most impactful, with an estimated total intake of around 39–52 thousand per person per year [12,13]. Once ingested/inhaled, MPs can be internalised in human tissues [11]. At the gastrointestinal level, they may pass through the epithelium by endocytosis mechanisms or by paracellular diffusion, after which they are translocated by dendritic cells through the lymphatic circulation and reach the circulatory system [11]. As regards the respiratory system, inhaled MPs likely penetrate the lower respiratory tract, characterised by a thin mucus layer, and spread into the bloodstream after cellular uptake or paracellular diffusion [45].

Currently, there is growing scientific evidence about MPs in humans. Schwabl et al. reported the detection of MPs in human stool [46], while, as a further measure, Ibrahim et al. described the presence of MPs in human colectomy samples, proving that MPs in part cross the intestinal barrier [47]. As evidence of inhalation exposure, Amato-Lourenço et al. detected, in human lung tissue, <5.5 µm polymeric MPs and fibres ranging from 8.12 to 16.8 µm [48]. We recently found MPs in the human placenta, which represents the interface between the foetus and the mother exposed to the external environment [21], results that were also confirmed by Braun et al. [49]. Very recently, the presence of plastic particles in human blood finally proved the transport of MPs in the bloodstream to every body site [50].

As regards the mammary gland, two hypothetical pathways have been suggested for the translocation of exogenous particles from the bloodstream to breast milk: the mammary epithelial cell-dependent and the immune cell-dependent pathways, with the latter being particularly relevant in the case of inhaled particles [23–25,51,52]. Hence, a possible association between the presence of MPs in breastmilk and specific information regarding mothers' habits (such as the consumption of fish and shellfish, beverages in plastic bottles, and food in plastic packaging and the use of personal care products containing plastic compounds in the 7 days prior to the expected date of delivery and the 7 days after) was

investigated, but no relationship was found between MP presence/number and each of the above-mentioned parameters.

The lack of association with the use of personal care products is likely explained by considering that dermal contact has a minor impact as an exposure route, since only particles <100 nanometres can cross the dermal barrier [11]. Conversely, the absence of a relation with mothers' food habits is more difficult to explain, since the major route of MP exposure is represented by ingestion. In fact, numerous food-related sources of MPs have been reported, including fish, shellfish, and human essential daily consumables, such as table salt, sugar, bottled water, milk, honey, plastic teabags, and to a greater extent, plastic kitchen tools, plates, and packaging [53]. Hence, our findings suggest that, since MPs are ubiquitous in the environment, exposure to these microparticles is inevitable, and, for this reason, it is impossible to isolate a specific source among the complex set of faced exposures.

5. Conclusions

The evidence of MPs in human breastmilk, coupled with the previous discovery of these microparticles in the human placenta, represents a great concern, since it impacts the extremely vulnerable population of infants. In fact, the chemicals possibly contained in foods, beverages, and personal care products consumed by breastfeeding mothers may be transferred to the offspring, potentially exerting a toxic effect. Hence, it is mandatory to increase efforts in scientific research to deepen the knowledge of the potential health impairment caused by MP internalisation and accumulation, especially in infants, and to assess innovative, useful ways to reduce exposure to these contaminants during pregnancy and lactation.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Safer Choice Standard and Criteria

Safer Choice Standard | Ingredient Criteria | Product-Class Criteria

Safer Choice Standard

- **Read the most recent version of the Safer Choice Standard**
<<https://epa.gov/saferchoice/safer-choice-standard>> **(February 2015)**
- Overview of the standard
- Changes to the standard
- Implementation and compliance schedules
- Frequently asked questions (FAQs) about ingredient disclosure, packaging, and performance requirements

Overview

The Safer Choice Standard, formerly known as DfE's Standard for Safer Products (or the "DfE Standard") identifies the requirements products and their ingredients must meet to earn the Safer Choice label.


Read the most recent version of the Safer Choice Standard <<https://epa.gov/saferchoice/safer-choice-standard>> (February 2015)

Changes to the Safer Choice Standard

The Safer Choice Standard was revised in February 2015. The Safer Choice Standard now includes provisions for the Safer Choice label, an associated fragrance-free label, and changes to the standard to implement the Safer Choice label.

Read the Changes to the Standard to Implement the Safer Choice Label

<<https://epa.gov/saferchoice/safer-choice-standard-2015-update>>.

Read the Notice of Availability and Request for Comments 

<<http://www.regulations.gov/docket/epa-hq-oppt-2015-0047>>.

Implementation and compliance schedules

To ensure your products are compliant with the most recent version of the Safer Choice Standard, please read the Safer Choice Implementation and Compliance Schedules

<<https://epa.gov/saferchoice/safer-choice-implementation-and-compliance-schedules>>.

Frequently asked questions (FAQs) about ingredient disclosure, packaging, and performance requirements

- Ingredient disclosure FAQs <https://epa.gov/sites/default/files/2015-02/documents/ingredient_disclosure_guidance.pdf>
- Packaging FAQs <https://epa.gov/sites/default/files/2015-02/documents/packaging_guidance.pdf>
- Product performance FAQs <https://epa.gov/system/files/documents/2022-04/product_performance_guidance.pdf>

Criteria for Safer Chemical Ingredients

Each chemical ingredient in a formulation has a function in making a product work - whether it is to aid in cleaning by reducing surface tension (surfactants), dissolve or suspend materials (solvents), or reduce water hardness (chelating agents). Within these "functional classes," many ingredients share similar toxicological and environmental fate characteristics. As a result, Safer Choice focuses its review of formulation ingredients on the key (environmental and human health) characteristics of concern within a functional class. This approach allows formulators to use those ingredients with the lowest hazard in their functional class, while still formulating high-performing products.

The Safer Choice Program evaluates each ingredient in a formulation against the following Master and Functional-Class Criteria documents, as appropriate. These documents define the characteristics and toxicity thresholds for ingredients that are acceptable in Safer Choice products.

The criteria are based on EPA expertise in evaluating the physical and toxicological properties of chemicals, and while they incorporate authoritative lists of chemicals of concern, they go far beyond these lists. Safer Choice applies the criteria using EPA research and analytical methods to ensure that Safer Choice products contain only the safest possible ingredients. All criteria documents are part of the Safer Choice Standard.

- Master criteria <<https://epa.gov/saferchoice/safer-choice-master-criteria-safer-chemical-ingredients>>
- Functional-class criteria
 - Chelating and sequestering agents <<https://epa.gov/saferchoice/safer-choice-criteria-chelating-and-sequestering-agents>>
 - Colorants, polymers, preservatives, and related chemicals <<https://epa.gov/saferchoice/safer-choice-criteria-colorants-polymers-preservatives-and-related-chemicals>>
 - Defoamers <<https://epa.gov/saferchoice/safer-choice-criteria-defoamers>>
 - Enzymes and enzyme stabilizers <<https://epa.gov/saferchoice/safer-choice-criteria-enzymes-and-enzyme-stabilizers>>
 - Fragrances <<https://epa.gov/saferchoice/safer-choice-criteria-fragrances>>
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 - Processing aids and additives <<https://epa.gov/saferchoice/safer-choice-criteria-processing-aids-and-additives>>
 - Solvents <<https://epa.gov/saferchoice/safer-choice-criteria-solvents>>
 - Surfactants <<https://epa.gov/saferchoice/safer-choice-criteria-surfactants>>

Safer Choice Product-Class Criteria

In addition to the product and ingredient criteria in the Safer Choice Standard, supplemental requirements are necessary to ensure that certain classes of products achieve best-in-class status and qualify to carry the Safer Choice label. These supplemental requirements are set forth in the criteria documents below.

- Direct release products
- Fragrance-free products
- Ice-melt products
- Inorganic- and mineral-based products
- Microorganism-based products
- Personal care products
- Safer marine lubricants
- Specialized industrial products

Criteria for environmental toxicity and fate for chemicals in direct release products

Certain products intended for use outdoors are likely to bypass sewage treatment, limiting the time for degradation prior to entering sensitive environments. For these products, like boat cleaners and graffiti removers, Safer Choice has raised the bar in its standard environmental criteria to address the potential for immediate contact with aquatic life. **Any ingredients (including surfactants, preservatives, solvents, etc.) that have aquatic toxicity values <10 mg/L are not allowed in Safer Choice direct release products.**

Supplemental criteria for chemicals in direct release products

Acute Aquatic Toxicity Value (L/E/IC50)	Chronic Aquatic Toxicity Value (LOEC)	Persistence ¹ (measured in terms of rate of biodegradation)	Bioaccumulation Potential	Status
---	---------------------------------------	--	---------------------------	--------

If acute aquatic	OR chronic			Not
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toxicity Acute ≤10 Aquatic ppm Toxicity	toxicity Chronic ≤1 Aquatic ppm Toxicity	⇒ Persistence¹ (measured in terms of rate of biodegradation)	⇒ Bioaccumulation Potential	acceptable Status
Value (L/E/IC50)	Value (LOEC)	biodegradation²		
If acute aquatic toxicity >10 ppm and <100 ppm...	AND chronic aquatic toxicity >1 ppm...	occurs within a 10-day window without degradation products of concern ³AND BCF/BAF <1,000 ⁴ ...	Acceptable
		...AND biodegradation ² does not occur within a 10-day window and/or results in degradation products of concern ³ ...	⇒	Not acceptable

		...AND biodegradation ²		
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Acute Aquatic Toxicity Value (L/E/IC50)	Chronic Aquatic Toxicity Value (LOEC)	occurs within 28 days without persistence¹ (measured in terms of rate of biodegradation) products of concern³...	...AND BCF/BAF <1,000⁴... Bioaccumulation Potential	Acceptable Status
toxicity ≥ 100 ppm...	toxicity ≥ 10 ppm...	...AND biodegradation ² does not occur within 28 days and/or results in degradation products of concern ³ ...	⇒	Not acceptable

1. Insoluble, inert, metal-containing, or inorganic compounds may be inherently persistent and are therefore considered recalcitrant, meaning resistant to degradation or elemental. For direct release applications, recalcitrant chemicals may be acceptable if they are measured or predicted to have low aquatic toxicity; both acute (≥ 100 ppm) and chronic (≥ 10 ppm) values will be considered.
2. Generally, >60% mineralization (to CO₂ and water) in a Ready Biodegradation test.
3. Products of concern are compounds with high acute or chronic aquatic toxicity (L/E/IC₅₀ ≤ 10 ppm or LOEC ≤ 1 ppm) and a slow rate of biodegradation (greater than 28 days).
4. Category for Persistent, Bioaccumulative and Toxic New Chemical Substance. November 4, 1999. Federal Register Notice, volume 64, issue 213.

Criteria for fragrance-free products



From Safer Choice Standard Section 3.9 (pg. 11) <<https://epa.gov/saferchoice/safer-choice-standard>>: "For products that qualify for the Safer Choice label, manufacturers may request an additional certification—the Fragrance-free label—to indicate that a product contains no fragrance materials. To qualify as fragrance-free, a product must only contain ingredients on or eligible for the Agency's Safer Chemical Ingredients List (SCIL) and **must not** contain any fragrance materials. Chemicals with dual functionality, i.e., that function both as a fragrance and something else, are not allowed in fragrance-free products."

Please read "Safer Choice Label – Fragrance-Free" for further details

<<https://epa.gov/saferchoice/safer-choice-criteria-fragrance-free-products>>.

Criteria for ice-melt products

An ice-melt product under Safer Choice is, as the name implies, one that melts ice and snow at temperatures below the freezing point of water, and not simply a product that aids traction like sand. A manufacturer of a safer ice-melt product may become a Safer Choice partner provided that they agree to certain terms in their partnership agreement and that their product has the characteristics specified below. Safer Choice ice-melt products must:

1. Pass the appropriate Safer Choice Criteria.
 2. Reduce sodium (Na) and chloride (Cl) use by at least 30% (under comparable use scenarios).
 3. Be labeled under a Safer Choice partnership agreement in which the product manufacturer has agreed to a customer education/training plan to ensure proper product use and application rates (and reductions in Na and Cl).
 4. Not contain cyanide as an anti-caking agent.
 5. Function at temperatures <0 °F.
 6. Comply with Pacific NW Snow Fighters' criteria 
<http://onlinepubs.trb.org/onlinepubs/nchrp/nchrp_rpt_577.pdf> (pdf) for reduction in corrosivity to steel (to be acceptable, a corrosion-inhibition chemical product must prove to have a percent effectiveness value of at least 70% less than Sodium Chloride).
 7. Meet performance levels as evaluated under the Pacific NW Snow Fighters' criteria  <http://onlinepubs.trb.org/onlinepubs/nchrp/nchrp_rpt_577.pdf> (pdf).
-

Criteria for inorganic- and mineral-based products

To label innovative, safer products, the Safer Choice review focuses on the evaluation of wet-chemical ingredients and formulations. Safer Choice assesses ingredients based on its safer chemical criteria and in comparison to other products/ingredients for similar uses. The Safer Choice Criteria inform on what chemistry is safer by comparing

substances, within functional use classes (surfactants, solvents, etc.), against an array of toxicological endpoints. Safer Choice allows the use of its label on those products whose ingredients derive entirely from the safer end of the human health and environmental spectrum.

The standard Safer Choice review is not oriented to evaluating a product composed solely of inorganic materials or minerals, which are typically inert and function via friction rather than chemical activity. Safer Choice recognizes, however, that these products may substitute for chemical-based products that contain ingredients of potential concern and may generate significant direct and collateral human health and environmental benefits. Safer Choice has therefore developed evaluation criteria that may make it possible to label these products (e.g., cleaners made of crushed glass or stones; **not**, however, sodium-chloride-based or similar ice-melt products for which Safer Choice has separate criteria).

A decision to allow use of the Safer Choice label will be based on the following criteria (in addition to the other applicable elements in the Safer Choice Standard):

1. Hazard profile.

- The hazard profile of the inorganic or mineral materials: Ingredients must not raise any toxicological concerns. Consider, for example, if the material is asbestiform or fibrous, or if potential impurities are present (e.g., problematic metals or crystalline silica).
- The hazard profile of the non-mineral-based ingredients: Ingredients must pass the appropriate Criteria for Safer Chemical Ingredients.

2. Recycled content. Product must be composed of at least 95% recycled materials. If the product includes a plastic handle or other plastic part, it must also be reusable and/or composed of at least 95% recycled content.

3. Manufacturing/sustainability. Candidate partner must provide information and/or data to demonstrate that only permissible air, land or water releases occur during the product's manufacturing process (e.g., via environmental release permits or waste manifests). They must also report on energy and water use as indicia of resource conservation and a baseline for continuous improvement.

4. Safer substitution. Product must have the potential to meet the same functional need as chemical-based products currently in use.

5. **Exposure and release.** Product in use must not generate particles that are inhalable (10 microns or less). - Product must not produce potential waste products of concern (candidate must submit an analysis of the byproducts generated).
 6. **Packaging.** Packaging must comply with the Safer Choice Standard, section 4.2.6. Any paperboard in the packaging should be made of or work toward 100% recycled content.
 7. **Performance.** Product must perform well in comparison to a leading brand without damaging surfaces.
-

Criteria for microorganism-based products

Microorganism-based products are a distinct class and subject to tailored evaluation criteria. In its review, Safer Choice carefully considers the identity and potential hazards and risks of the microbial species, as informed by its Checklist for Formulations Containing Microorganisms <<https://epa.gov/saferchoice/safer-choice-criteria-formulations-containing-microorganisms>>, in combination with other considerations like purity of strain, ingredient functionality and product performance, as described in its Considerations for Microorganism-based Products <<https://epa.gov/saferchoice/safer-choice-criteria-formulations-containing-microorganisms>>.

Please note: microbial-based products intended for use in indoor environments are not eligible for partnership. Non-microbial ingredients will be reviewed based on their respective component-class criteria.

Criteria for personal care products (PCP)

In April 2011, Safer Choice finalized section 4.5 of the Safer Choice Standard: “Products Designed for Dermal Contact.” With the increased interest in the Safer Choice label from personal care product manufacturers, the question has arisen whether all personal care products should be reviewed under section 4.5.

Safer Choice intended that the heightened requirements in section 4.5, developed in consultation with the Food and Drug Administration, would apply only to certain classes of personal care products:

- those that are “leave-on” in nature and result in prolonged dermal contact, like lotions and deodorants, and
- those that, regardless of length of exposure, are made to come into contact with infants and children, whose bodies are developing and particularly sensitive to certain chemicals.

In both cases, formulators may address the restriction on sensitizers (including ingredients that lack sensitization data) by listing them on the product label or by providing whole product sensitization testing.

The following matrix categorizes personal care products into two groups based on whether section 4.5 applies or does not apply. While Safer Choice has reviewed and labeled rinse-off personal care products, like hand soaps, Safer Choice has yet to label a leave-on product subject to section 4.5.

Section 4.5 applies (leave-on PCP)	Section 4.5 doesn't apply (rinse-off PCP)
Aftershave	Body wash
Astringent/toner	Bubble bath and bath salts
Cleaning wipes that don't require rinsing after use	Hair conditioner
Cuticle cream, lotion, and oil	Exfoliant products (if rinsed off)
Deodorant and antiperspirant	Face wash
Hair shine products	Hair dye, color, and bleach
Hair spray	Hair relaxants
Hair styling products (e.g., balm, gel, mousse)	Makeup remover (if rinsed off)

Section 4.5 applies (leave-on PCP)	Section 4.5 doesn't apply (rinse-off PCP)
Leave-on hair conditioner	Moisturizing products (if rinsed off)
Lip products	Nail polish remover
Makeup and bronzers (e.g., foundation, concealer, bronzer, mascara, eyeliner, eye shadow, blush)	Shampoo
Makeup remover (if left on)	Shaving cream, gel, and foam
Massage oil	Soap and cleansers
Nail polish	
Skin care products (e.g., lotion, moisturizer, cream, oil, serum)	
Sunless tanning products	

Criteria for safer marine lubricants

Manufacturers of marine lubricants subject to the Office of Water Vessel General Permit (VGP) requirements for environmentally acceptable lubricants (EALs), who wish to qualify for the Safer Choice label, must comply with the Safer Choice Standard and Criteria, with the limited exceptions and additional requirements specified below.

It is noteworthy that the chemicals in marine lubricants typically include as part of their functionality the ability to resist degradation and be effective over long periods under adverse conditions. These chemicals also can be complex molecules and mixtures and often lack measured toxicity data. To identify the safest available chemicals given their functional characteristics, the toxicity thresholds in the Safer Choice Master Criteria

<<https://epa.gov/saferchoice/safer-choice-master-criteria-safer-chemical-ingredients>> will be used to evaluate human health endpoints, and the thresholds below will be used for environmental endpoints.

A. **Human and environmental health requirements.** Candidate products for EAL marine lubricant status must meet, at a minimum, the following ingredient data and hazard limit requirements:

- a. *For acute mammalian toxicity (section 5.1 of the Master Criteria), neurotoxicity (5.4), repeated dose toxicity (5.5), respiratory sensitization (5.7), and skin sensitization (5.8), the following data requirements apply:*

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists for each chemical present in a mixture. Chemicals with new data not yet reviewed by authoritative bodies will be subject to review

- b. *For carcinogenicity (section 5.2 of the Master Criteria), genetic toxicity (5.3), and reproductive and developmental toxicity (5.6), the following data requirements apply:*

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists. All available data, measured and/or estimated, for the chemical or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

c. Environmental toxicity and fate

Limitations on persistent, bioaccumulative and toxic chemicals: Acceptable chemicals must not be persistent (half-life >60 days), bioaccumulative (BCF/BAF =1,000), and aquatically toxic* (LC/EC50 <10 mg/L or NOEC/LOEC <1 mg/L).

Limitation on very persistent and very bioaccumulative chemicals: Acceptable chemicals must not be very persistent (half-life >180 days or recalcitrant) and very aquatically toxic* (LC/EC50 <1.0 mg/L or NOEC/LOEC <0.1 mg/L).

Limitation on very persistent and very toxic chemicals: Acceptable chemicals must not be very persistent (half-life >180 days or recalcitrant) and very aquatically toxic* (LC/EC50 <1.0 mg/L or NOEC/LOEC <0.1 mg/L).

Data requirements: Screen specified R-Phrases, H-Phrases, and Authoritative Lists. All available data, measured and/or estimated, for the chemical or a suitable analog will be reviewed against the criteria using a weight-of-evidence approach.

B. Direct environmental release. Ingredients in lubricants that are intended for use in applications that result in their immediate discharge to the environment, bypassing sewage treatment systems, must meet the Criteria for Environmental Toxicity and Fate for Chemicals in Direct Release Products, based on the biodegradation testing in OECD 306.

C. Renewable content^[1]. Products must meet the following renewable content requirements:

- a. Hydraulic fluid, transmission fluid, gear oil, and grease: at least 65 percent.
- b. Two-stroke oil: at least 50 percent.

D. Performance. Products must demonstrate acceptable performance. For example, pass the ASTM D 665 test - "Standard Test Method for Rust Preventing Characteristics of Inhibited Mineral Oil in the Presence of Water."

Criteria for specialized industrial products

Specialized Industrial Products (SIPs) are a distinct subgroup of products that meet tailored criteria under the Safer Choice Program. Safer Choice is using the term "specialized" for this subset of I/I products to distinguish them based on performance requirements from other, more common I/I products, like cleaners and detergents, and to indicate that they require certain ingredients with special, high-performance functionalities. Nevertheless, to earn the Safer Choice label, a candidate product and its ingredients must meet the general SIP criteria, in section II, as well as the subclass-specific requirements, in section III.

Read the Criteria for Specialized Industrial Products <<https://epa.gov/saferchoice/safer-choice-criteria-specialized-industrial-products>>.

[1] *Renewable content* means the use of farm- or agriculture-based products, like vegetable oils and animal fats.

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[How to List on SCIL <https://epa.gov/saferchoice/how-list-chemical-safer-chemical-ingredients-list>](https://epa.gov/saferchoice/how-list-chemical-safer-chemical-ingredients-list)

Safer Choice Standard and Criteria

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CHAPTER 9 - Degradation of Polymers

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Publisher Summary

This chapter focuses on approaches employed for the degradation of polymers. Environmental pollution by synthetic polymers, such as waste plastics and water-soluble synthetic polymers in wastewater has been recognized as a major problem. Degradation of polymers can be carried out by heat, radiation, or biochemical treatment. The radiant energy may be high-energy radiation from gamma rays, ion beams, and electrons or even low-energy radiation from ultraviolet (UV) light. UV stabilizers added to polymer products reduce the rate of degradation. Chemical degradation results from treatment with chemicals such as acids and alkalis. Biodegradation of polymers results from the use of microorganisms and enzymes. Since most of the polymers are resistant to degradation, research over the past several years has focused on developing biodegradable polymers that are degraded and ultimately catabolized to carbon dioxide and water by bacteria and fungi under natural conditions. During the degradation process, they should not generate any substances that are harmful. These polymers can be classified into three major categories: polyesters produced by microorganisms, natural polysaccharides and other biopolymers like starch, and synthetic polymers like aliphatic polymers. Another approach toward achieving biodegradability has been through the addition of biodegradable groups into the main chain during the production of industrial polymers prepared by free radical copolymerization. Two such approaches are the use of ethylene bis(mercaptoacetate) as a chain transfer agent during the copolymerization of styrene and MMA, and the preparation of copolymers of vinylic monomers with cyclic comonomers containing the biodegradable functions such as ketene acetal and cyclic disulfides.



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Plastic pollution is growing relentlessly as waste management and recycling fall short, says OECD

22/02/2022 - The world is producing twice as much plastic waste as two decades ago, with the bulk of it ending up in landfill, incinerated or leaking into the environment, and only 9% successfully recycled, according to a new OECD report.

Ahead of UN talks on international action to reduce plastic waste, the OECD's first **Global Plastics Outlook** shows that as rising populations and incomes drive a relentless increase in the amount of plastic being used and thrown away, policies to curb its leakage into the environment are falling short.

Almost half of all plastic waste is generated in OECD countries, according to the Outlook. Plastic waste generated annually per person varies from 221 kg in the United States and 114 kg in European OECD countries to 69 kg, on average, for Japan and Korea. Most plastic pollution comes from inadequate collection and disposal of larger plastic debris known as macroplastics, but leakage of microplastics (synthetic polymers smaller than 5 mm in diameter) from things like industrial plastic pellets, synthetic textiles, road markings and tyre wear are also a serious concern.

OECD countries are behind 14% of overall plastic leakage. Within that, OECD countries account for 11% of macroplastics leakage and 35% of microplastics leakage. The Outlook notes that international co-operation on reducing plastic pollution should include supporting lower-income countries in developing better waste management infrastructure to reduce their plastic leakage.

The report finds that the COVID-19 crisis led to a 2.2% decrease in plastics use in 2020 as economic activity slowed, but a rise in littering, food takeaway packaging and plastic medical equipment such as masks has driven up littering. As economic activity resumed in 2021, plastics consumption has also rebounded.

Reducing pollution from plastics will require action, and international co-operation, to reduce plastic production, including through innovation, better product design and developing environmentally friendly alternatives, as well as efforts to improve waste management and increase recycling.

Bans and taxes on single-use plastics exist in more than 120 countries but are not doing enough to reduce overall pollution. Most regulations are limited to items like plastic bags, which make up a tiny share of plastic waste, and are more effective at reducing littering than curbing plastics consumption. Landfill and incineration taxes that incentivise recycling only exist in a minority of countries. The Outlook calls for greater use of instruments such as Extended Producer Responsibility schemes for packaging and durables, landfill taxes, deposit-refund and Pay-as-You-Throw systems.

Most plastics in use today are virgin – or primary – plastics, made from crude oil or gas. Global production of plastics from recycled – or secondary – plastics has more than quadrupled from 6.8 million tonnes (Mt) in 2000 to 29.1 Mt in 2019, but this is still only 6% of the size of total plastics production. More needs to be done to create a separate and well-functioning market for recycled plastics, which are still viewed as substitutes for virgin plastic. Setting recycled content targets and investing in improved recycling technologies could help to make secondary markets more competitive and profitable.

Some key findings from the Outlook:

- Plastic consumption has quadrupled over the past 30 years, driven by growth in emerging markets. Global plastics production doubled from 2000 to 2019 to reach 460 million tonnes. Plastics account for 3.4% of global greenhouse gas emissions.
- Global plastic waste generation more than doubled from 2000 to 2019 to 353 million tonnes. Nearly two-thirds of plastic waste comes from plastics with lifetimes of under five years, with 40% coming from packaging, 12% from consumer goods and 11% from clothing and textiles.

- Only 9% of plastic waste is recycled (15% is collected for recycling but 40% of that is disposed of as residues). Another 19% is incinerated, 50% ends up in landfill and 22% evades waste management systems and goes into uncontrolled dumpsites, is burned in open pits or ends up in terrestrial or aquatic environments, especially in poorer countries.
- In 2019, 6.1 million tonnes (Mt) of plastic waste leaked into aquatic environments and 1.7 Mt flowed into oceans. There is now an estimated 30 Mt of plastic waste in seas and oceans, and a further 109 Mt has accumulated in rivers. The build-up of plastics in rivers implies that leakage into the ocean will continue for decades to come, even if mismanaged plastic waste could be significantly reduced.
- Considering global value chains and trade in plastics, aligning design approaches and the regulation of chemicals will be key to improving the circularity of plastics. An international approach to waste management should lead to all available sources of financing, including development aid, being mobilised to help low and middle-income countries meet estimated costs of EUR 25 billion a year to improve waste management infrastructure.

Access the [**OECD Global Plastics Outlook Database**](#)

Watch a presentation of the report: [**Green Talk Live**](#)

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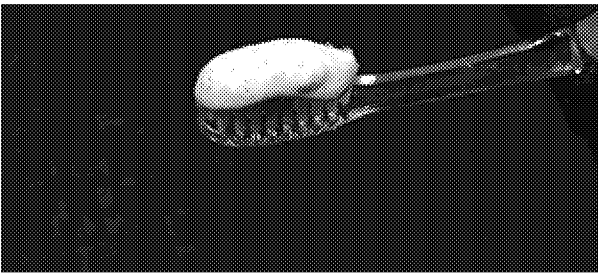
For further information, journalists are invited to contact [Catherine Bremer](#) in the OECD Media Office (+33 1 45 24 80 97).

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Also Available

廃棄物の管理とリサイクルが不十分で、プラスチック汚染の拡大が止まらない


La contaminación por plástico crece sin cesar, en tanto que la gestión de residuos y el reciclaje se quedan cortos, dice la OCDE.



Microbeads are tiny pieces of polyethylene plastic added to health and beauty products, such as some cleansers and toothpastes.

As an emerging field of study, not a lot is known about microplastics and their impacts yet. The NOAA Marine Debris Program is leading efforts within NOAA to research this topic. Standardized field methods for collecting sediment, sand, and surface-water microplastic samples have been developed and continue to undergo testing. Eventually, field and laboratory protocols will allow for global comparisons of the amount of microplastics released into the environment, which is the first step in determining the final distribution, impacts, and fate of this debris.

Microplastics come from a variety of sources, including from larger plastic debris that degrades into smaller and smaller pieces. In addition, microbeads, a type of microplastic, are very tiny pieces of manufactured polyethylene plastic that are added as exfoliants to health and beauty products, such as some cleansers and toothpastes. These tiny particles easily pass through water filtration systems and end up in the ocean and Great Lakes, posing a potential threat to aquatic life.

Microbeads are not a recent problem. According to the [United Nations Environment Programme](#) , plastic microbeads first appeared in personal care products about fifty years ago, with plastics increasingly replacing natural ingredients. As recently as 2012, this issue was still relatively unknown, with an abundance of products containing plastic microbeads on the market and not a lot of awareness on the part of consumers.

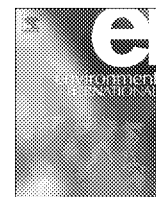
On December 28, 2015, President Obama signed the [Microbead-Free Waters Act of 2015](#) (<https://www.gpo.gov/fdsys/pkg/BILLS-114hr1321enr/pdf/BILLS-114hr1321enr.pdf>), banning plastic microbeads in cosmetics and personal care products.

Video Transcript

Plastic is everywhere. A lot of it ends up in the ocean. Most plastics in the ocean break up into very small particles. These small plastic bits are called "microplastics." Other plastics are intentionally designed to be small. They're called microbeads and are used in many health and beauty products. They pass unchanged through waterways into the ocean. Aquatic life and birds can mistake microplastics for food. Research is being conducted. But there's still much we don't know. In 2015, the U.S. banned the use of microbeads. But microplastics are still a huge problem. You can help keep plastic out of the ocean. Remember: Reduce. Reuse. Recycle.

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Plasticenta: First evidence of microplastics in human placenta

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ABSTRACT

Microplastics are particles smaller than five millimeters deriving from the degradation of plastic objects present in the environment. Microplastics can move from the environment to living organisms, including mammals. In this study, six human placentas, collected from consenting women with physiological pregnancies, were analyzed by Raman Microspectroscopy to evaluate the presence of microplastics. In total, 12 microplastic fragments (ranging from 5 to 10 μm in size), with spheric or irregular shape were found in 4 placentas (5 in the fetal side, 4 in the maternal side and 3 in the chorioamniotic membranes); all microplastics particles were characterized in terms of morphology and chemical composition. All of them were pigmented; three were identified as stained polypropylene a thermoplastic polymer, while for the other nine it was possible to identify only the pigments, which were all used for man-made coatings, paints, adhesives, plasters, finger paints, polymers and cosmetics and personal care products.

1. Introduction

In the last century, the global production of plastics has reached 320 million tons (Mt) per year, and over 40% is used as single-use packaging, hence producing plastic waste. In Europe, plastic production reached the 58 millions of tons in 2014 (PlasticsEurope, 2016). The degradation that plastics undergo when released into the environment is a serious issue. Atmospheric agents, such as waves, abrasion, ultraviolet radiation and photo-oxidation in combination with bacteria degrade plastic fragments into micro and nanosized particles. Most of the seabed all over the world and in the Mediterranean Sea in particular, is made of plastic, resulting from the waste collected on the coasts and in the sea (de Souza Machado et al., 2018). Microplastics (MPs) are defined as particles less than 5 mm in size (Hartmann et al., 2019). MPs do not derive only from larger pieces fragmentation but are also produced in these dimensions for commercial uses. Furthermore, there are several reports of MPs in food (Barboza et al., 2018), and in particular in seafood, sea salt (Karimi et al., 2017b; Kosuth et al., 2018), and in drinking water (Schymanski

et al., 2018). Microplastics have also been detected in the gastrointestinal tract of marine animals (Deng et al., 2017; Reineke et al., 2013), and also human intestine (Schwabl et al., 2019). Inside tissues, MPs are considered as foreign bodies by the host organism and, as such, trigger local immunoreactions. Furthermore, MPs can act as carriers for other chemicals, such as environmental pollutants and plastic additives, which may be released and are known for their harmful effects (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016; Wright and Kelly, 2017).

In this study, for the first time, several microplastic fragments were detected by Raman Microspectroscopy in human placenta samples collected from six consenting patients with uneventful pregnancies. Raman Microspectroscopy is a well-assessed vibrational technique, widely and successfully applied in the biomedical field, to characterize both biological samples (Notarstefano et al., 2020, 2019), and to detect the occurrence of microplastics and microparticles in general (Käppler et al., 2016; Ribeiro-Claro et al., 2017). Placenta finely regulates foetal to maternal environment and, indirectly, to the external one, acting as a

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crucial interface via different complex mechanisms (PrabhuDas et al., 2015). The potential presence of man-made MPs in this organ may harm the delicate response of differentiation between self and non-self (Nancy et al., 2012) with a series of related consequences on embryo development that need to be defined.

2. Materials and methods

2.1. Experimental design

This was a pilot observational descriptive preclinical study, with a prospective and unicentric open cohort. It was approved by the Ethical Committee Lazio 1 (Protocol N. 352/CE Lazio 1; March 31th, 2020), and it was carried out in full accordance with ethical principles, including The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. To participate to this study, six selected consenting patients signed an informed consent, which included donation of placentas.

To prevent plastic contamination, a plastic-free protocol was adopted during the entire experiment. Obstetricians and midwives used cotton gloves to assist women in labour. In the delivery room, only cotton towels were used to cover patients' beds; graduate bags to estimate postpartum blood loss were not used during delivery, but they

were brought in the delivery room only after birth, when umbilical cord was already clamped and cut with metal clippers, avoiding contact with plastic material. Pathologists wore cotton gloves and used metal scalpels.

The schematic illustration for the overall concept and experimental procedure is reported in Fig. 1.

2.2. Enrolment of patients and placentas collection

All recruited women were healthy and have a vaginal delivery at term of pregnancy at the Department of Obstetrics and Gynaecology of San Giovanni Calibita Fatebenefratelli Hospital, Isola Tiberina, Roma (Italy). They were selected according to the following exclusion criteria: diagnosis of gastrointestinal disease, such as ulcerative colitis, or Crohn's disease, cancer, organ transplantation, HIV (Human Immunodeficiency Virus), or other severe pathologies; alcohol abuse (defined as a >10 score in the Alcohol Use Disorders Identification Test); cigarette smoking; peculiar diets prescribed for particular medical conditions (four weeks before delivery); diarrhoea or constipation (two weeks before delivery); antibiotics intake (two weeks before delivery); assumption of drugs affecting intestinal reabsorption, such as activated charcoal, or cholestyramine (two weeks before delivery); invasive or abrasive dental treatments (two weeks before delivery); participation to

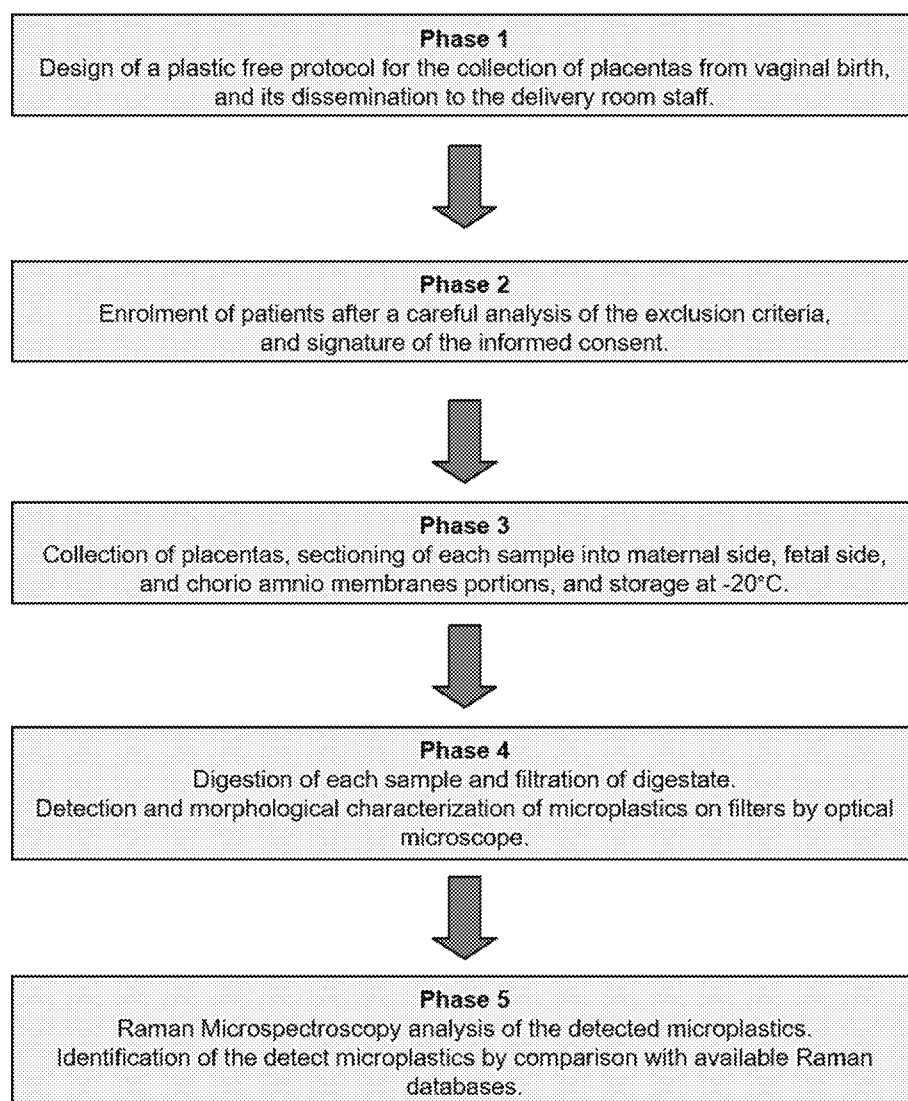


Fig. 1. Schematic illustration for the overall concept and experimental procedure followed in the study.

a clinical study (four weeks before delivery). Women were also asked to fill a questionnaire to record their food consumption (omnivorous, vegetarian, vegan, with no diet restriction) the week before delivery and the use of toothpastes and cosmetics containing MPs or synthetic polymers.

After birth, placentas were deposited onto a metal container and immediately sectioned in portions (mean weight: 23.3 ± 5.7 g) taken from maternal side, foetal side, and chorioamniotic membranes. All samples were strictly anonymous; they were labelled with number codes and stored in glass bottles with metal lids at -20 °C with no further treatment.

2.3. Digestion of placenta samples

The digestion of placenta samples was performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy), modifying as follows the protocols from two previous works (Dehaut et al., 2016; Karimi et al., 2017a). Samples were weighed and placed in a glass container. A 10% KOH solution was prepared using $1.6 \mu\text{m}$ -filtered deionised water and KOH tablets (Sigma-Aldrich). This solution was added to each jar in a ratio with the sample of 1:8 (w/v). The containers were then sealed and incubated at room temperature for 7 days. To prevent plastic contamination, cotton laboratory coats, face masks and single-use latex gloves were worn during sample handling, preparation of samples and during the entire experiment. Work surfaces were thoroughly washed with 70% ethanol prior starting all procedures. All liquids (deionised water for cleaning and for preparation of KOH solution) were filtered through $1.6 \mu\text{m}$ -pore-size filter membrane (Whatman GF/A). Glassware and instruments, including scissors, tweezers and scalpels, were washed using dishwashing liquid, rinsed with deionised water and finally rinsed with $1.6 \mu\text{m}$ -filtered deionised water. Since the experiments were conducted without the use of the laminar flow hood, the plastic fibres found in the samples were not considered in the results. Digestates were then filtered through $1.6 \mu\text{m}$ -pore-size filter membrane (Whatman GF/A) using a vacuum pump connected to a filter funnel. The filter papers were dried at room temperature and stored in glass Petri dishes until visual identification and spectroscopic characterization of plastic particles. Three procedural blanks, obtained following the same procedure above described, but without placenta samples and maintained close to the samples during their manipulation, were tested to monitor and correct potential contaminations (Karimi et al., 2017a).

2.4. Analysis of microplastics by Raman Microspectroscopy

The Raman analysis of MPs was performed at the Laboratory of Vibrational Spectroscopy, Department of Life and Environmental Sciences, Università Politecnica delle Marche (Ancona, Italy). Filter membranes were first inspected by visible light using a $\times 10$ objective (Olympus MPLAN10x/0.25). The detected MPs were morphologically characterized by a $\times 100$ objective (Olympus MPLAN100x/0.90), and then directly analyzed on the filter by Raman Microspectroscopy (spectral range $160\text{--}2000 \text{ cm}^{-1}$, 785 nm laser diode, 600 lines per mm grating). A Raman XploRA Nano Microspectrometer (Horiba Scientific) was used. The spectra were dispersed onto a 16-bit dynamic range Peltier cooled CCD detector; the spectrometer was calibrated to the 520.7 cm^{-1} line of silicon prior to spectral acquisition. Raw Raman spectra were submitted to polynomial baseline correction and vector normalization, in order to reduce noise and enhance spectrum quality (Labspec 6 software, Horiba Scientific). The collected Raman spectra were compared with those reported in the SLOPP Library of Microplastics ("SLOPP Library of Microplastics," n.d.) and in the spectral library of the KnowItAll software (Bio-Rad Laboratories, Inc.). Similarities of more than 80 of Hit Quality Index (HQI) were considered satisfactory.

3. Results

From each placenta, three portions with a mean weight of 23.3 ± 5.7 g were collected from the maternal side, the foetal side and the chorioamniotic membranes. All portions were opportunely processed for the subsequent analysis by Raman Microspectroscopy.

In total, 12 MP fragments (named #1–#12) were detected in the placentas of four women; more in detail, 5 MPs were found in the foetal side portions, 4 in the maternal side portions, and 3 in the chorioamniotic membranes. Microplastics #1–#4, #6, #7, and #9–#12, were $\sim 10 \mu\text{m}$ in size, while #5 and #8 ones were smaller ($\sim 5 \mu\text{m}$). All the analyzed MPs were pigmented.

A retrospective analysis based on Raman spectral information and data reported in literature was performed to define the nature of these MPs. Firstly, the collected Raman spectra were compared with those stored in the spectral library of the KnowItAll software (Bio-Rad Laboratories, Inc.). In many cases, the collected Raman spectra showed, above all, the contribution of the pigments used for plastic staining (Imhof et al., 2016; Stoye and Freitag, 1998); it is known that the conjugated rings present in pigment molecules are highly polarizable and, hence, their Raman signals are more intense than those of the apolar polymeric matrix (Käppler et al., 2016). In these cases, the KnowItAll software identified the pigments contained in the MPs. By matching the results from the KnowItAll software with the information obtained by consulting the European Chemical Agency, ECHA ("European Chemical Agency," n.d.), it was possible to accurately identify the commercial name, chemical formula, IUPAC name and Color Index Constitution Number of all pigments. Further, in order to uncover the identity of the polymer matrix of the detected MPs, the collected Raman spectra were also compared with those reported in the SLOPP library of Microplastics.

The identified MPs were differentiated between stained MPs (particles #2, #10 and #11, identified as polypropylene) and paint/coating/dye MPs (particles #1, #3–9, and #12), which are applied for paints, coatings, adhesives, plasters, polymers and cosmetics and personal care products (Imhof et al., 2016).

The microphotographs and the Raman spectra of all analysed MPs are shown in Fig. 2, while Table 1 reports their morphological and chemical characterization. The spectral analysis is reported below.

Particle #1 (Fig. 2a): the Raman spectrum resulted superimposable to the one of the pigment Iron hydroxide oxide yellow (main peak at 396 cm^{-1} , related to the vibrations of iron oxides/hydroxides) (Skute et al., 2018).

Particles #2, #5, and #10 (Fig. 2b, e): the Raman spectra resulted comparable to the one of a polypropylene (PP) blue sample, sharing the main peaks at 253 cm^{-1} (wagging of CH_2 moieties, bending of CH moieties), 397 cm^{-1} (wagging of CH_2 moieties, bending of CH moieties), 839 cm^{-1} (rocking of CH_2 and CH_3 moieties, stretching of CC and C- CH_3 moieties), 970 cm^{-1} (rocking of CH_3 moieties), and 1455 cm^{-1} (bending of CH_3 and CH_2 moieties), all assigned to PP (Andreassen, 1999). The bands at 679 cm^{-1} , 1143 cm^{-1} , 1340 cm^{-1} and 1527 cm^{-1} , common to reference blue polypropylene and sample spectra, are known to be related to Raman signals of blue pigments, mainly based on copper phthalocyanine (Aguayo et al., 2010; Scherrer et al., 2009).

Particle #3 (Fig. 2c): the Raman spectrum resulted superimposable to the one of the blue pigment phthalocyanine, sharing the main peaks at 679 cm^{-1} , 1143 cm^{-1} , 1340 cm^{-1} and 1527 cm^{-1} (Aguayo et al., 2010; Scherrer et al., 2009).

Particle #4 (Fig. 2d): the Raman spectrum resulted superimposable to the one of the pigment violanthrone, with the two main peaks centered at 1577 cm^{-1} (C-C stretching of benzene ring) and 1307 cm^{-1} (in reference spectrum, an additional shoulder at $\sim 1350 \text{ cm}^{-1}$ is visible, assigned to C-C stretching and HC-C bending) (Socrates, 2001).

Particles #6 and #7 (Fig. 2f): the Raman spectra resulted superimposable to the one of the red pigment oxo(oxoferriooxy)iron (main peaks at 220 , 287 and 401 cm^{-1} , typical of iron oxides) (Testa-Anta et al., 2019).

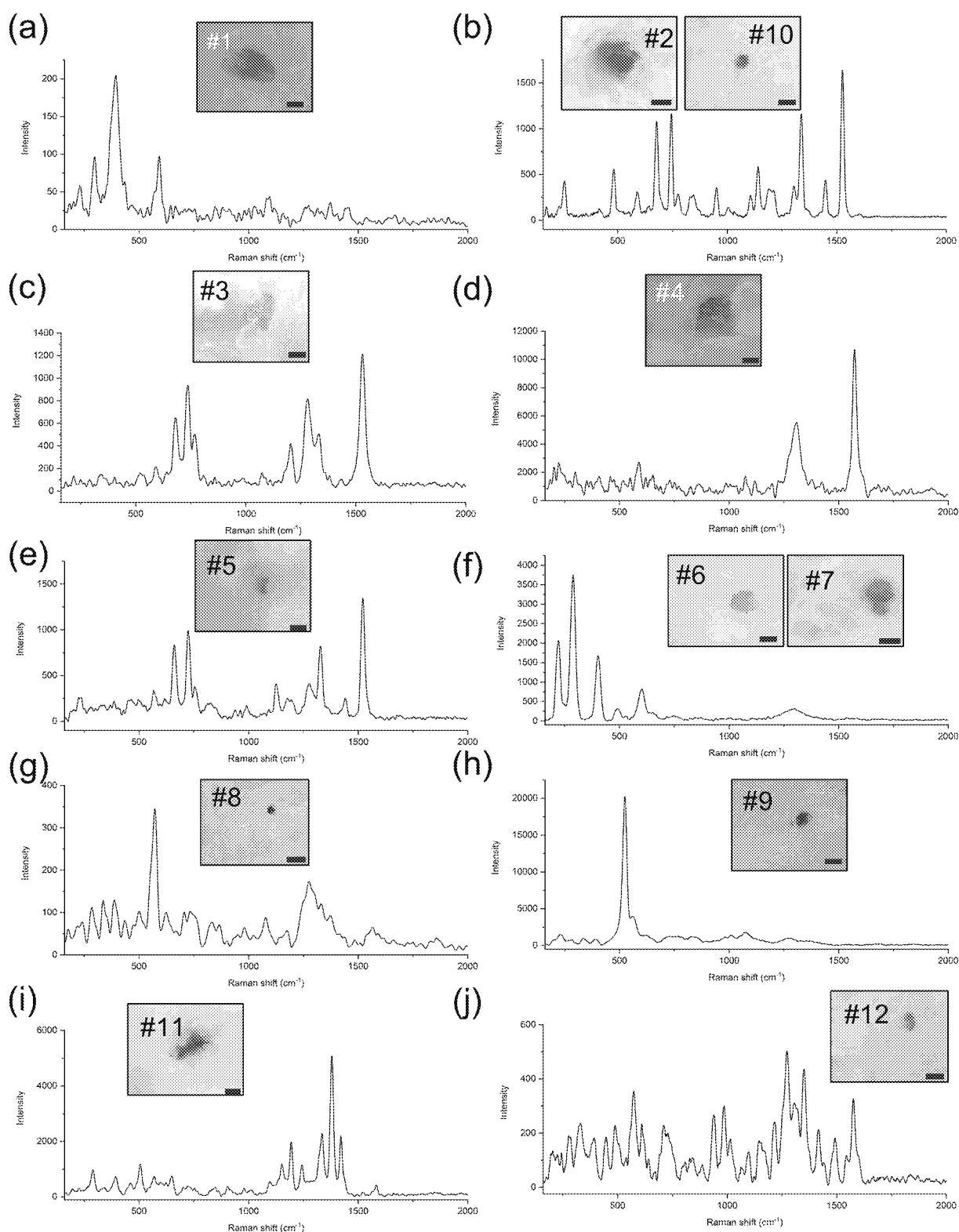


Fig. 2. Microphotographs and Raman spectra of the microplastics found in human placenta: (a) Particle #1 (scale bar 5 μm); (b) Particles #2 and #10 (scale bar 5 μm for #2 and 10 μm for #10); (c) Particle #3 (scale bar 5 μm); (d) Particle #4 (scale bar 5 μm); (e) Particle #5 (scale bar 5 μm); (f) Particles #6 and #7 (scale bar 10 μm for #6 and 5 μm for #7); (g) Particle #8 (scale bar 10 μm); (h) Particle #9 (scale bar 10 μm); (i) Particle #11 (scale bar 5 μm), and (l) Particle #12 (scale bar 10 μm).

Particle #8 (Fig. 2g): the Raman spectrum resulted superimposable to the one of the pigment Direct Blue 80, showing two main peaks at 578 cm^{-1} and 1275 cm^{-1} (both assigned to ring deformations of substituted benzene) (Socrates, 2001).

Particle #9 (Fig. 2h): the Raman spectrum resulted superimposable

to the one of the pigment Ultramarine Blue, with one main peak centred at 525 cm^{-1} , assigned to orthosilicate vibration modes (Socrates, 2001).

Particle #11 (Fig. 2i): the Raman spectrum resulted comparable to the one of a PP purple fiber, sharing the main peaks of PP (Andreassen, 1999) (397 cm^{-1} , assigned to the wagging of CH_2 moieties/bending of

Table 1

Size, color and chemical features of the detected microplastics and relative pigments, together with information regarding the placenta portion in which they were found (fetal side FS; maternal side MS, and chorioamnio membrane CAM; not defined n.d.; Hit Quality Index HQI).

Particle	Placenta Portion	Microparticles			Pigment		HQI
		Size	Color	Polymer matrix	Generic name	Molecular formula and IUPAC name	
#1	FS	~10 μm	Orange	n.d.	Iron hydroxide oxide yellow (Pigment Yellow 43; C.I. Constitution 77492)	$\text{FeO}(\text{OH})$ iron(III) oxide hydroxide	89.97
#2	CAM	~10 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$\text{C}_{32}\text{H}_{16}\text{CuN}_8$ (29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32)copper(II)	82.86
#3	FS	~10 μm	Blue	n.d.	Phthalocyanine Blue BN (Pigment Blue 16; C.I. Constitution 74100)	$\text{C}_{32}\text{H}_{18}\text{N}_8$ 29H,31H-phthalocyanine	89.16
#4	MS	~10 μm	Dark blue	n.d.	Violanthrone (Pigment Blue 65; C.I. Constitution 59800)	$\text{C}_{34}\text{H}_{16}\text{O}_2$ Anthra[9,1,2-cde]benzo[1st]pentaphene-5,10-dione	86.44
#5	MS	~5 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$\text{C}_{32}\text{H}_{16}\text{CuN}_8$ (29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32)copper(II)	86.15
#6	MS	~10 μm	Red	n.d.	Diiron trioxide (Pigment Red 101/102; C.I. Constitution 77491)	Fe_2O_3 Oxo(oxoferriooxy)iron	83.65
#7	MS	~10 μm	Red	n.d.	Diiron trioxide (Pigment Red 101/102; C.I. Constitution 77491)	Fe_2O_3 Oxo(oxoferriooxy)iron	89.80
#8	CAM	~5 μm	Dark blue	n.d.	Pigment Direct Blue 80	$\text{C}_{32}\text{H}_{14}\text{Cu}_2\text{N}_4\text{Na}_4\text{O}_{16}\text{S}_4$ Dicopper,tetrasodium,3-oxido-4-[[2-oxido-4-[3-oxido-4-[[2-oxido-3,6-disulfonatophthalen-1-yl]diazenyl]phenyl]phenyl]diazenyl]naphthalene-2,7-disulfonate	84.55
#9	CAM	~10 μm	Dark blue	n.d.	Ultramarine Blue (Pigment Blue 29; C.I. Constitution 77007)	$\text{Al}_6\text{Na}_8\text{O}_{24}\text{S}_{16}$ Aluminium Sodium orthosilicate trisulfane-1,3-diide	91.96
#10	FS	~10 μm	Blue	Polypropylene	Copper phthalocyanine (Pigment Blue 15; C.I. Constitution 74160)	$\text{C}_{32}\text{H}_{16}\text{CuN}_8$ (29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32)copper(II)	80.60
#11	FS	~10 μm	Violet	Polypropylene	Hostopen violet (Pigment Violet 23; C.I. Constitution 51319)	$\text{C}_{34}\text{H}_{22}\text{Cl}_2\text{N}_4\text{O}_2$ 8,18-Dichloro-5,15-diethyl-5,15-dihydroindolo(3,2-b:3',2'-m)tri-phenodioxazine	80.92
#12	FS	~10 μm	Pink	n.d.	Novoperm Bordeaux HF3R (Pigment Violet 32; C.I. Constitution 12517)	$\text{C}_{27}\text{H}_{24}\text{N}_6\text{O}_7\text{S}$ 4-[(E)-2-[2,5-dimethoxy-4-(methylsulfamoyl)phenyl]diazen-1-yl]-3-hydroxy-N-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)naphthalene-2-carboxamide	84.57

CH moieties, and 1455 cm^{-1} , assigned to the bending of CH_3 and CH_2 moieties), and also of the violet pigment (1193 cm^{-1} , 1335 cm^{-1} and 1381 cm^{-1}) (Scherrer et al., 2009).

Particle #12 (Fig. 2j): the collected Raman spectrum resulted superimposable to the one of the pink pigment Novoperm Bordeaux HF3R. The Raman spectrum of this monoazopigment shared with the sample spectrum the main peaks centered at 731 cm^{-1} , 961 cm^{-1} , 1219 cm^{-1} , 1280 cm^{-1} , 1360 cm^{-1} , and 1580 cm^{-1} . This pigment is reported to be used to permanently coat and protect wood surfaces, in photographic chemicals, inks and toners, given its high solvent resistance and good heat stability (Scherrer et al., 2009).

4. Discussion

This is the first study revealing the presence of pigmented microplastics and, in general, of man-made particles in human placenta. The presence of pigments in all analysed MPs is explained by the wide use of these compounds to colour not only plastic products, but also paints and coatings, which are as ubiquitous as MPs (Imhof et al., 2016). For example, the pigment Iron hydroxide oxide yellow (particle #1) is used for coloration of polymers (plastics and rubber) and in a wide variety of cosmetics, such as BB creams and foundations; copper phthalocyanine (particles #2, #5, #10,) and phthalocyanine (particle #3) are used for staining of plastic materials (polyvinylchloride, low density polyethylene, high density polyethylene, polypropylene, polyethylene terephthalate), and for finger paints; the pigment violanthrone (particle #4) is used especially for textile (cotton/polyester) dyeing, coating products, adhesives, fragrances and air fresheners; the pigment Ultramarine blue is mainly applied in cosmetics, for example for formulations of soap, lipstick, mascara, eye shadow and other make-up products.

For the first time, by means of Raman Microspectroscopy, 12 MP fragments were isolated in four human placentas. In particular, 5 MPs

were found in the foetal side, 4 in the maternal side and 3 in the chorioamniotic membranes, indicating that these MPs, once inside the human body, can reach placenta tissues at all levels. It is noteworthy to remark that small portions of placentas (~23 g with respect to a total weight of ~600 g) were analysed, letting hypothesize that the number of MPs within the entire placenta is much higher.

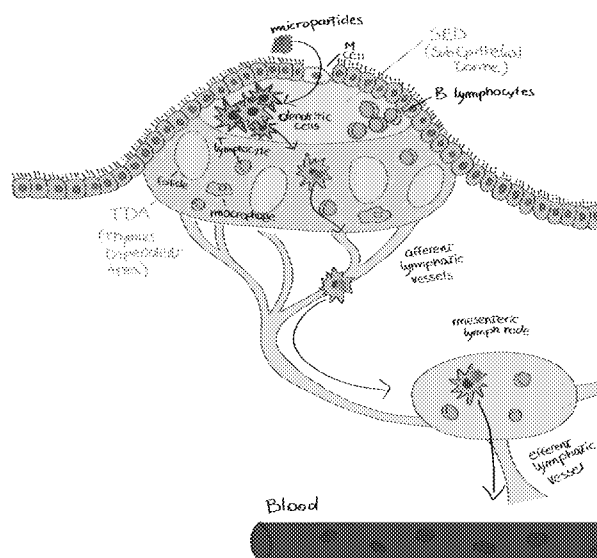
The dimensions of all MPs were ~10 μm in size, except for two that were smaller (~5 μm). These values are compatible with a possible transportation by bloodstream. In fact, previous analyses performed by means of Electron Microscopy coupled with an X-ray microprobe, revealed the presence of 5–10 μm particles as foreign bodies in human internal organs (Vaseashta, 2015).

Unfortunately, we do not know how MPs reach the bloodstream and if they come from the respiratory system or the gastrointestinal system. Fig. 3 shows the possible ways of entry and transport of the MPs from the respiratory and gastric organs to the placenta.

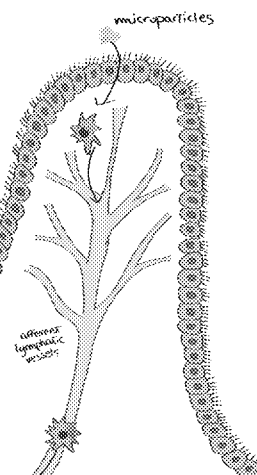
The presence of MPs in the placenta tissue requires the reconsideration of the immunological mechanism of self-tolerance. Placenta represents the interface between the foetus and the environment (PrabhuDas et al., 2015). Embryos and fetuses must continuously adapt to the maternal environment and, indirectly, to the external one, by a series of complex responses. An important part of this series of responses consists in the ability to differentiate self and non-self (Nancy et al., 2012), a mechanism that may be perturbed by the presence of MPs. In fact, it is reported that, once present in the human body, MPs may accumulate and exert localized toxicity by inducing and/or enhancing immune responses and, hence, potentially reducing the defence mechanisms against pathogens and altering the utilization of energy stores (Wright and Kelly, 2017).

Microplastics may access the bloodstream and reach placenta from the maternal respiratory system (Schlesinger, 1983) and the gastrointestinal tract (GIT) (Arumugasaamy et al., 2019), by means of M cells-

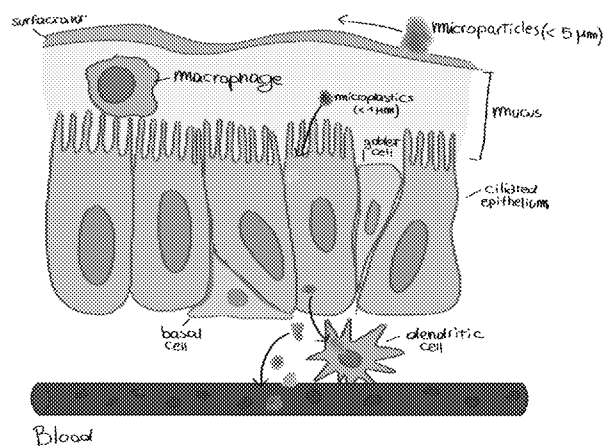
A) Endocytosis by T Cells



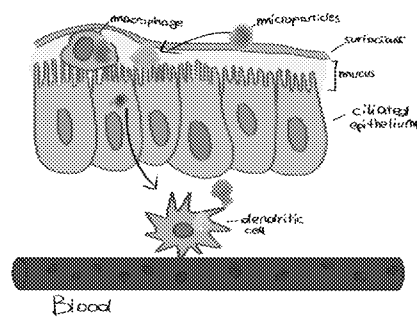
B) Paracellular Diffusion



C) Upper Airways



D) Lower Airways



(caption on next page)

Fig. 3. A-B-C-D. Hypothetical mechanisms by which microplastics penetrate human tissues. (A) Endocytosis by M cells. At the level of the Peyer's Patch, below the mucous gut, MPs ingested with food can be uptaken by endocytosis from the M cells, transported across the epithelium into the subepithelial dome where they encounter dendritic cells, which in turn transport them through the lymphatic circulation, from where they reach the blood. (B) Paracellular Diffusion. MPs could penetrate through the intestinal lumen from loose junctions. This phenomenon could partially explain why some inflammatory states, which increase loose junctions favour intestinal passage. Once the intestinal lumen has been crossed, the MPs are collected by the dendritic cells and transported in the lymphatic and subsequently in the systemic circulation. (C) Upper airways. At the level of the upper respiratory tract the mucus is thicker and allows a successful clearance of the foreign bodies particles, in addition, the mechanical movement of ciliated epithelium and the presence of surfactant prevents smaller particles from spreading through the epithelium and reach the circulation. (D) Lower airways. In the lower respiratory tract the mucus layer is thinner, thus facilitating the diffusion of particles which, thanks to their particular aerodynamic shape, are able to reach this part of the respiratory tract. Once penetrated, the MPs can spread into the general circulation by cellular uptake or diffusion. (Modified from: Mowat, A. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 3, 331–341 (2003). <https://doi.org/10.1038/nri1057>. And Ruge, C. A.; Kirch, J.; Lehr, C. M. Pulmonary drug delivery: From generating aerosols to overcoming biological barriers-therapeutic possibilities and technological challenges. *Lancet. Respir. Med.* 2013, 1(5), 402–413.)

mediated endocytosis mechanisms or paracellular transport. The most probable transport route for MPs is a mechanism of particle uptake and translocation, already described for the internalization from the GIT (Smith et al., 2018). The subsequent translocation to secondary target organs, usually associated with inflammatory responses in the surrounding tissues, such as the immune activation of macrophages and the production of cytokines (Hicks et al., 1996), depends on several factors, including hydrophobicity, surface charge, surface functionalization and the associated protein corona, and particle size.

Once MPs have reached the maternal surface of the placenta, as other exogenous materials, they can invade the tissue in depth by several transport mechanisms, both active and passive, that are not clearly understood yet (Tetro et al., 2018). The transplacental passage of 5–10 µm size MPs may depend on different physiological conditions and genetic characteristics. This might explain, together with the diverse food habits and lifestyle of patients, the absence of MPs in 2 of the 6 analyzed placentas and the different localization and characteristics of the particles identified in the present study. It is known that a great variability exists in the expression and function of placental drug transporters, both within human populations (inter-individual variability) and also during gestation (intra-individual variability) (Staud and Ceckova, 2015). We suppose that this variability exists also in relation to the mechanism of particles' internalization.

Potentially, MPs, and in general microparticles, may alter several cellular regulating pathways in placenta, such as immunity mechanisms during pregnancy, growth-factor signalling during and after implantation, functions of atypical chemokine receptors governing maternal-foetal communication, signalling between the embryo and the uterus, and trafficking of uterine dendritic cells, natural killer cells, T cells and macrophages during normal pregnancy. All these effects may lead to adverse pregnancy outcomes including preeclampsia and fetal growth restriction (Ilekis et al., 2016).

In conclusion, this study sheds new light on the level of human exposure to MPs and microparticles in general. Due to the crucial role of placenta in supporting the foetus development and in acting as an interface between the latter and the external environment, the presence of exogenous and potentially harmful (plastic) particles is a matter of great concern. Possible consequences on pregnancy outcomes and foetus are the transgenerational effects of plasticizer on metabolism and reproduction (Lee, 2018). Further studies need to be performed to assess if the presence of MPs in human placenta may trigger immune responses or may lead to the release of toxic contaminants, resulting harmful for pregnancy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Microplastics collected from the Magothy River in Maryland.

WILL PARSON/CHESAPEAKE BAY PROGRAM

MICROPLASTICS ARE EVERYWHERE — BUT ARE THEY HARMFUL?

Scientists are rushing to study the tiny plastic specks that are in marine animals — and in us.

By XiaoZhi Lim

Dunzhu Li used to microwave his lunch each day in a plastic container. But Li, an environmental engineer, stopped when he and his colleagues made a disturbing discovery: plastic food containers shed huge numbers of tiny specks — called microplastics — into hot water. “We were shocked,” Li says. Kettles and baby bottles also shed microplastics, Li and other researchers, at Trinity College Dublin,

reported last October¹. If parents prepare baby formula by shaking it up in hot water inside a plastic bottle, their infant might end up swallowing more than one million microplastic particles each day, the team calculated.

What Li and other researchers don’t yet know is whether this is dangerous. Everyone eats and inhales sand and dust, and it’s not clear if an extra diet of plastic specks will harm us. “Most of what you ingest is going to pass straight through your gut and out the other

end,” says Tamara Galloway, an ecotoxicologist at the University of Exeter, UK. “I think it is fair to say the potential risk might be high,” says Li, choosing his words carefully.

Researchers have been worried about the potential harms of microplastics for almost 20 years — although most studies have focused on the risks to marine life. Richard Thompson, a marine ecologist at the University of Plymouth, UK, coined the term in 2004 to describe plastic particles smaller than 5 millimetres across, after his team found them on British beaches. Scientists have since seen microplastics everywhere they have looked: in deep oceans; in Arctic snow and Antarctic ice; in shellfish, table salt, drinking water and beer; and drifting in the air or falling with rain over mountains and cities. These tiny pieces could take decades or more to degrade fully. “It’s almost certain that there is a level of exposure in just about all species,” says Galloway.

The earliest investigations of microplastics focused on microbeads found in personal-care products, and pellets of virgin plastic that can escape before they are moulded into objects, as well as on fragments that slowly erode from discarded bottles and other large debris. All these wash into rivers and oceans: in 2015, oceanographers estimated there were between 15 trillion and 51 trillion microplastic particles floating in surface waters worldwide. Other sources of microplastic have since been identified: plastic specks shear off from car tyres on roads and synthetic microfibres shed

from clothing, for instance. The particles blow around between sea and land, so people might be inhaling or eating plastic from any source.

From limited surveys of microplastics in the air, water, salt and seafood, children and adults might ingest anywhere from dozens to more than 100,000 microplastic specks each day, Albert Koelmans, an environmental scientist at Wageningen University in the Netherlands, reported this March². He and his colleagues think that in the worst cases, people might be ingesting around the mass of a credit card's worth of microplastic a year.

Regulators are taking the first step towards quantifying the risk to people's health – measuring exposure. This July, the California State Water Resources Control Board, a branch of the state's environmental protection agency, will become the world's first regulatory authority to announce standard methods for quantifying microplastic concentrations in drinking water, with the aim of monitoring water over the next four years and publicly reporting the results.

Evaluating the effects of tiny specks of plastic on people or animals is the other half of the puzzle. This is easier said than done. More than 100 laboratory studies have exposed animals, mostly aquatic organisms, to microplastics. But their findings – that exposure might lead some organisms to reproduce less effectively or suffer physical damage – are hard to interpret because microplastics span many shapes, sizes and chemical compositions, and many of the studies used materials that were quite unlike those found in the environment.

The tiniest specks, called nanoplastics – smaller than 1 micrometre – worry researchers most of all (see 'Microplastics to scale'). Some might be able to enter cells, potentially

disrupting cellular activity. But most of these particles are too small for scientists even to see; they were not counted in Koelmans' diet estimates, for instance, and California will not try to monitor them.

One thing is clear: the problem will only grow. Almost 400 million tonnes of plastics are produced each year, a mass projected to more than double by 2050. Even if all plastic production were magically stopped tomorrow, existing plastics in landfills and the environment – a mass estimated at around 5 billion tonnes – would continue degrading into tiny fragments that are impossible to collect or clean up, constantly raising microplastic levels. Koelmans calls this a "plastic time bomb".

"If you ask me about risks, I am not that frightened today," he says. "But I am a bit concerned about the future if we do nothing."

Modes of harm

Researchers have several theories about how plastic specks might be harmful. If they're small enough to enter cells or tissues, they might irritate just by being a foreign presence – as with the long, thin fibres of asbestos, which can inflame lung tissue and lead to cancer. There's a potential parallel with air pollution: sooty specks from power plants, vehicle

exhausts and forest fires called PM₁₀ and PM_{2.5} – particulate matter measuring 10 µm and 2.5 µm across – are known to deposit in the airways and lungs, and high concentrations can damage respiratory systems. Still, PM₁₀ levels are thousands of times higher than the concentrations at which microplastics have been found in air, Koelmans notes.

The larger microplastics are more likely to exert negative effects, if any, through chemical toxicity. Manufacturers add compounds such as plasticizers, stabilizers and pigments to plastics, and many of these substances are hazardous – for example, interfering with endocrine (hormonal) systems. But whether ingesting microplastics significantly raises our exposure to these chemicals depends on how quickly they move out of the plastic specks and how fast the specks travel through our bodies – factors that researchers are only beginning to study.

Another idea is that microplastics in the environment might attract chemical pollutants and then deliver them into animals that eat the contaminated specks. But animals ingest pollutants from food and water anyway, and it's even possible that plastic specks, if largely uncontaminated when swallowed, could help to remove pollutants from animal guts. Researchers still can't agree on whether pollutant-carrying microplastics are a significant problem, says Jennifer Lynch, a marine biologist affiliated with the US National Institute of Standards and Technology in Gaithersburg, Maryland.

Perhaps the simplest mode of harm – when it comes to marine organisms, at least – might be that organisms swallow plastic specks of no nutritional value, and don't eat enough food to survive. Lynch, who also leads the Center for Marine Debris Research at Hawaii Pacific University in Honolulu, has autopsied sea turtles that are found dead on beaches, looking at plastics in their guts and chemicals in their tissues. In 2020, her team completed a set of analyses for 9 hawksbill turtle hatchlings, under 3 weeks old. One hatchling, only 9 centimetres long, had 42 pieces of plastic in its gastrointestinal tract. Most were microplastics.

"We don't believe any of them died specifically from plastics," Lynch says. But she wonders whether the hatchlings might have struggled to grow as fast as they need to. "It's a very tough stage of life for those little guys."

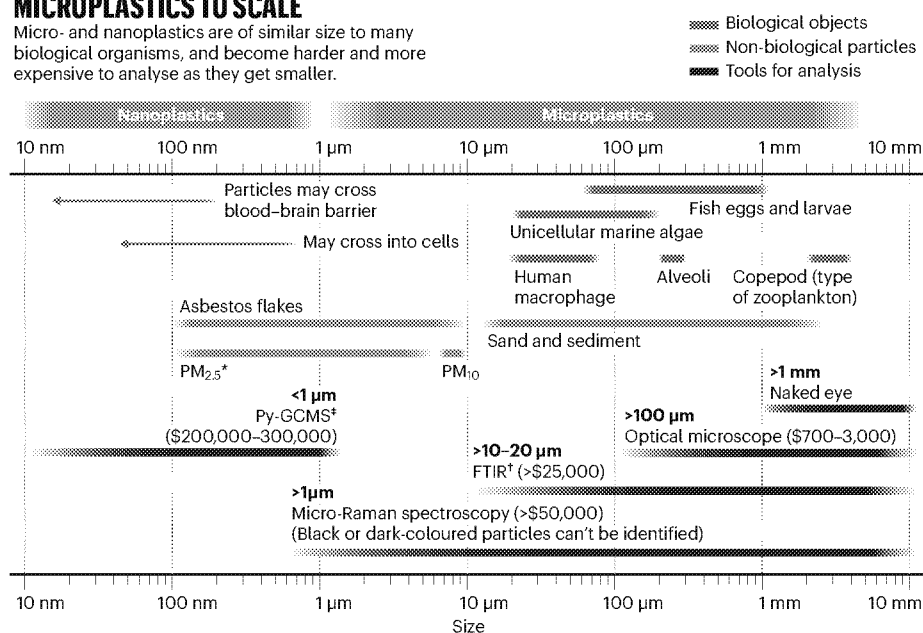
Marine studies

Researchers have done the most work on microplastic risks to marine organisms. Zooplankton, for instance, among the smallest marine organisms, grow more slowly and reproduce less successfully in the presence of microplastics, says Penelope Lindeque, a marine biologist at the Plymouth Marine Laboratory, UK: the animals' eggs are smaller and less likely to hatch. Her experiments show

"It's almost certain that there is a level of exposure in just about all species."

MICROPLASTICS TO SCALE

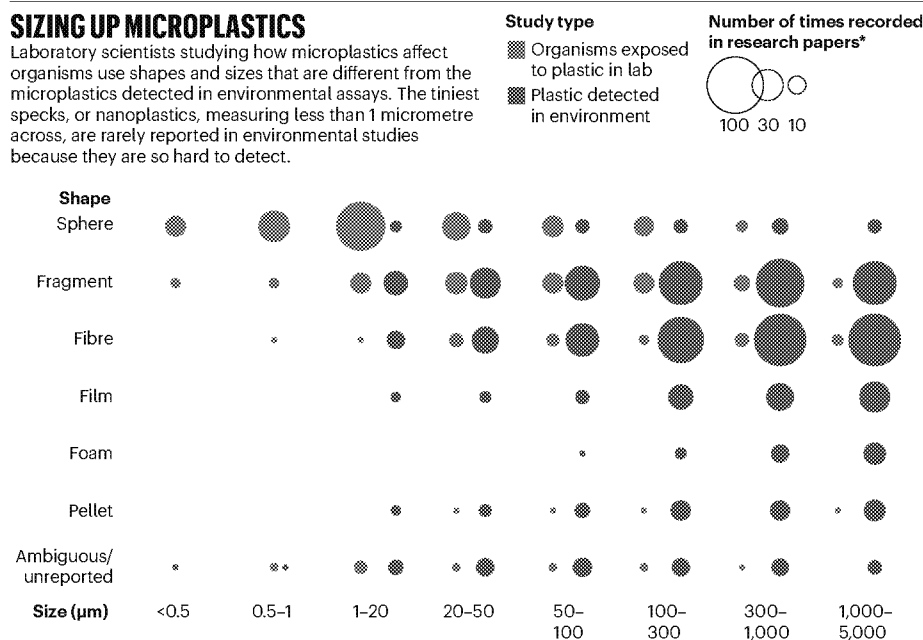
Micro- and nanoplastics are of similar size to many biological organisms, and become harder and more expensive to analyse as they get smaller.



*Particulate matter less than 2.5 micrometres (PM_{2.5}) or less than 10 µm (PM₁₀) in diameter, often from soot, vehicle exhaust or dust; [†]FTIR, Fourier-transform infrared spectroscopy; [‡]Py-GCMS, pyrolysis-gas chromatography-mass spectrometry.

SIZING UP MICROPLASTICS

Laboratory scientists studying how microplastics affect organisms use shapes and sizes that are different from the microplastics detected in environmental assays. The tiniest specks, or nanoplastics, measuring less than 1 micrometre across, are rarely reported in environmental studies because they are so hard to detect.



*Nature analysis of 136 detection and 159 exposure studies.

that the reproduction problems stem from the zooplankton not eating enough food³.

But, because ecotoxicologists started running experiments before they knew what kinds of microplastics exist in aquatic environments, they depended heavily on manufactured materials, typically using polystyrene spheres of smaller sizes and at concentrations much higher than surveys found (see 'Sizing up microplastics').

Scientists have started shifting to more environmentally realistic conditions and using fibres or fragments of plastics, rather than spheres. Some have started coating their test materials in chemicals that mimic biofilms, which appear to make animals more likely to eat microplastics.

Fibres seem to be a particular problem. Compared with spheres, fibres take longer to pass through zooplankton, Lindeque says. In 2017, Australian researchers reported that zooplankton exposed to microplastic fibres produced half the usual number of larvae and that the resulting adults were smaller. The fibres were not ingested, but the researchers saw that they interfered with swimming, and identified deformations in the organisms' bodies⁴. Another study⁵ in 2019 found that adult Pacific mole crabs (*Emerita analoga*) exposed to fibres lived shorter lives.

Most laboratory studies expose organisms to one type of microplastic, of a specific size, polymer and shape. In the natural environment, organisms are exposed to a mixture, says Koelmans. In 2019, he and his doctoral student Merel Kooi plotted the abundances of microplastics reported from 11 surveys of oceans, rivers and sediment, to build models of mixtures in aquatic environments.

Last year, the two teamed up with colleagues to use this model in computer simulations

that predict how often fish would encounter microplastics small enough to eat, and the likelihood of eating enough specks to affect growth. The researchers found that at current microplastic pollution levels, fish run that risk at 1.5% of locations checked for microplastics⁶. But there are likely to be hotspots where the risks would be higher, says Koelmans. One possibility is the deep sea: once there, and often buried in sediment, it is unlikely the microplastics will travel elsewhere and there is no way to clean them up.

The oceans already face many stressors, which makes Lindeque more afraid that microplastics will further deplete zooplankton populations than that they will transfer up the food chain to reach people. "If we knock out something like zooplankton, the base of our marine food web, we'd be more worried about impacts on fish stocks and the ability to feed the world's population."

Human studies

No published study has yet directly examined the effects of plastic specks on people, leading researchers say. The only available studies rely on laboratory experiments that expose cells or human tissues to microplastics, or use animals such as mice or rats. In one study⁷, for instance, mice fed large quantities of microplastics showed inflammation in their small intestines. Mice exposed to microplastics in two studies had a lowered sperm count⁸ and fewer, smaller pups⁹, compared with control groups. Some of the *in vitro* studies on human cells or tissues also suggest toxicity. But, just as with the marine studies, it's not clear that the concentrations used are relevant to what mice – or people – are exposed to. Most of the studies also used polystyrene spheres, which don't represent the diversity of microplastics that

people ingest. Koelmans also points out that these studies are among the first of their kind, and could end up being outliers once there's an established body of evidence. There are more *in vitro* studies than animal studies, but researchers say they still don't know how to extrapolate the effects of solid plastic specks on tissues to possible health problems in whole animals.

One question surrounding risk is whether microplastics could remain in the human body, potentially accumulating in some tissues. Studies in mice have found that microplastics around 5 µm across could stay in the intestines or reach the liver. Using very limited data on how quickly mice excrete microplastics and the assumption that only a fraction of particles 1–10 µm in size would be absorbed into the body through the gut, Koelmans and colleagues estimate that a person might accumulate several thousand microplastic particles in their body over their lifetime².

Some researchers have started to explore whether microplastics can be found in human tissue. In December, a team documented this for the first time in a study that looked at six placentas¹⁰. Researchers broke down the tissue with a chemical, then examined what was left, and ended up with 12 particles of microplastic in 4 of those placentas. Yet it's not impossible that these specks were the result of contamination when the placentas were collected or analysed, says Rolf Halden, an environmental-health engineer at Arizona State University in Tempe – although he commends the researchers for their efforts to avoid contamination, which included keeping delivery wards free of plastic objects, and for showing that a control set of blank materials taken through the same sample analysis was not contaminated. "There is a continuing challenge of demonstrating conclusively that a given particle actually originated in a tissue," he says.

Those who are worried by their microplastic exposure can reduce it, says Li. His work on kitchenware found that the amounts of plastic shed depend highly on temperature – which is why he's stopped microwaving food in plastic containers. To reduce issues with baby bottles, his team suggests that parents could rinse sterilized bottles with cool water that has been boiled in non-plastic kettles, so as to wash away any microplastics released during sterilization. And they can prepare baby formula in glass containers, filling feeding bottles after the milk has cooled. The team is now recruiting parents to volunteer samples of their babies' urine and stools for microplastic analysis.

The nano fraction

Particles that are small enough to penetrate and hang around in tissues, or even cells, are the most worrying kind, and warrant more attention in environmental sampling, says Halden. One study¹¹ that deliberately let

SOURCE: NATURE ANALYSIS

pregnant mice inhale extremely tiny particles, for instance, later found the particles in almost every organ in their fetuses. “From a risk perspective, that’s where the real concern is, and that’s where we need more data.”

To enter cells, particles generally need to be smaller than a few hundred nanometres. There was no formal definition of a nanoplastic until 2018, when French researchers proposed the upper size limit of 1 μm – tiny enough to remain dispersed through a water column where organisms can more easily consume them, instead of sinking or floating as larger microplastics do, says Alexandra ter Halle, an analytical chemist at Paul Sabatier University in Toulouse, France.

But researchers know almost nothing about nanoplastics; they are invisible and cannot simply be scooped up. Just measuring them has stumped scientists.

Researchers can use optical microscopes and spectrometers – which distinguish between particles by their differing interactions with light – to measure the length, width and chemical make-up of plastic particles down to a few micrometres. Below that scale, plastic particles become difficult to distinguish from non-plastic particles such as marine sediment or biological cells. “You’re looking for the needle in the haystack, but the needle looks like the hay,” says Roman Lehner, a nanomaterials scientist at the Sail and Explore Association, a Swiss non-profit research group.

In 2017, ter Halle and her colleagues proved for the first time that nanoplastic exists in an environmental sample: seawater collected from the Atlantic Ocean¹². She extracted colloidal solids from the water, filtered away any particles larger than 1 μm , burnt what remained, and used a mass spectrometer – which fragments molecules and sorts the fragments by molecular weight – to confirm that plastic

polymers had existed in the remnants.

That, however, gave no information on the exact sizes or shapes of the nanoplastics. Ter Halle got some idea by studying the surfaces of two degraded plastic containers she collected during the expedition. The top few hundred micrometres had become crystalline and brittle, she found; she thinks that this may also be true of the nanoplastics that probably broke off from these surfaces¹³. For now, because researchers cannot collect nanoplastics from the environment, those doing laboratory studies grind up their own plastic, expecting to get similar particles.

Using home-made nanoplastics has an advantage: researchers can introduce tags to help track the particles inside test organisms. Lehner and colleagues prepared fluorescent nano-sized plastic particles and placed them under tissue built from human intestinal-lining cells¹⁴. The cells did absorb the particles, but did not show signs of cytotoxicity.

“You’re looking for the needle in the haystack, but the needle looks like the hay.”

Finding plastic specks lodged in intact slices of tissue – through a biopsy, for instance – and observing any pathological effects would be the final piece of the puzzle over microplastic risks, Lehner says. This would be “highly desirable”, says Halden. But to reach tissues, the particles would have to be very small, so both researchers think it would be very

difficult to detect them conclusively.

Collecting all these data will take a lot of time. Ter Halle has collaborated with ecologists to quantify microplastic ingestion in the wild. Analysing only particles larger than 700 μm in some 800 samples of insects and fish took thousands of hours, she said. The researchers are now examining the particles in the 25–700 μm range. “This is difficult and tedious, and this is going to take a long time to get the results,” she says. To look at the smaller size range, she adds, “the effort is exponential.”

No time to lose

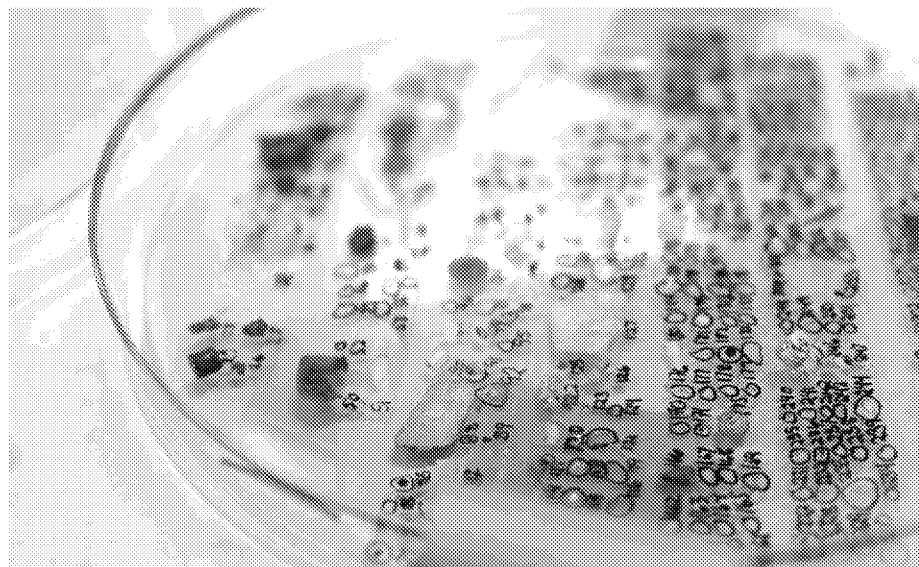
For the moment, levels of microplastics and nanoplastics in the environment are too low to affect human health, researchers think. But their numbers will rise. Last September, researchers projected¹⁵ that the amount of plastic added to existing waste each year – whether carefully disposed of in sealed landfills or strewn across land and sea – could more than double from 188 million tonnes in 2016 to 380 million tonnes in 2040. By then, around 10 million tonnes of this could be in the form of microplastics, the scientists estimated – a calculation that didn’t include the particles continually being eroded from existing waste.

It is possible to rein in some of our plastic waste, says Winnie Lau at the Pew Charitable Trusts in Washington DC, who is the first author on the study. The researchers found that if every proven solution to curb plastic pollution were adopted in 2020 and scaled up as quickly as possible – including switching to systems of reuse, adopting alternative materials, and recycling plastic – the amount of plastic waste added could drop to 140 million tonnes per year by 2040.

By far the biggest gains would come from cutting out plastics that are used only once and discarded. “There’s no point producing things that last for 500 years and then using them for 20 minutes,” Galloway says. “It’s a completely unsustainable way of being.”

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Microplastics collected in the San Francisco Bay area, labelled for study.



Biodegradation of poly (vinyl alcohol) based materials

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Abstract

Poly(vinyl alcohol) (PVA) is recognized as one of the very few vinyl polymers soluble in water also susceptible of ultimate biodegradation in the presence of suitably acclimated microorganisms. Accordingly, increasing attention is devoted to the preparation of environmentally compatible PVA-based materials for a wide range of applications. The present article is aimed at providing a survey of the available information on the environmental fate of PVA and PVA-based materials. Literature data and recent advances on the biochemistry and microbial physiology of PVA biodegradation and on the influence of environmental conditions are discussed along with the biodegradation processes of other water-soluble materials. The biodegradation behaviors of several PVA-based materials including blends, composites and copolymers are also reviewed and discussed.

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Keywords

Water-soluble; Poly(vinyl alcohol); Poly(vinyl acetate); Biodegradation; Blends; Composites

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